THE EFFICACY OF GLUFOSINATE AMMONIUM ON RYEGRASS AS INFLUENCED BY DIFFERENT PLANT GROWTH STAGES AND DIFFERENT TEMPERATURES

by

Tendai Mucheri

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Supervisor: Dr P.J Pieterse

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DECLARATION

I confirm that this Master's thesis is my own original work and I have documented all sources and material used. I declare that I am the authorship owner (unless to the extent explicitly stated). This thesis in its entirety or in part has not been previously submitted for obtaining any qualification.

Signature: …….....

Tendai Mucheri

Date: …….....

ABSTRACT

Herbicide resistance in weeds is the ability of weeds to survive and reproduce following exposure to the recommended dosage rate of herbicide that is lethal to its wild type. There is a widespread concern in agriculture about weeds with high genetic diversity that have developed resistance to weed control, ryegrass (*Lolium* spp.) included. Ryegrass has developed resistance to commonly used herbicides which include paraquat and glyphosate. There is an opportunity of using glufosinate ammonium to alleviate ryegrass weed resistance problems. The herbicide not only has a unique mode of action but also has no ryegrass resistance proven to it yet.

There are restrictive application timings with glufosinate ammonium since it is a contact herbicide. More specific recommended dosage rates of herbicides can therefore be developed by determining the contribution of environmental factors and growth stage of weeds to efficacy of glufosinate ammonium. The principle objective of the study was to determine the effective dosage of glufosinate ammonium for the control of ryegrass weed under different temperatures and ryegrass growth stages. Studies on the influence of temperature on glufosinate ammonium efficacy are reported in Chapter 3, 4 and 7. Influence of ryegrass growth stage on efficacy of glufosinate ammonium is dealt with in Chapters 5 and 6.

Glufosinate ammonium dosage rates of 0, 1.5, 3, 4.5, 6 and 7.5 L ha^{-1} were used for a temperature study in glasshouses running at 10/15, 15/20, 20/25, and 20/30 °C night/day temperatures. In Chapter 3 the influence of temperature on efficacy of glufosinate ammonium on young and mature ryegrass is described. Mature ryegrass was sprayed at 6 weeks while young ryegrass was sprayed at 3 weeks. The study proved that a low temperature of 10/15 °C controlled approximately 95% of both young and mature ryegrasses with 3 L ha^{-1} while the trend observed at 15/20 and 20/25 °C was irregular. Temperatures of 25/30 °C resulted in poor control of ryegrass. There was a general increase in control of young ryegrass as compared to mature ryegrass.

In Chapter 4 the effect of temperature on efficacy of glufosinate ammonium with the added adjuvant ammonium sulphate (AMS) on ryegrass is described. Applied glufosinate ammonium dosage rates were 1, 2 and 3 L ha^{-1} with added ammonium sulphate at rates 1, 2 and 3%. Glasshouses were set at 10/15, 15/20, 20/25, and 20/30 °C night/day temperatures. The findings of the study indicated that a dosage rate of 3 L ha^{-1} glufosinate ammonium with addition of 2 and 3% ammonium sulphate controlled ryegrass effectively. There was more

effective control of ryegrass with all concentrations of AMS at lower temperatures compared to the control at higher temperatures. An increase in AMS concentration resulted in an increase of ryegrass control at lower temperatures but this was not evident with control at higher temperature.

Glasshouse and field experiments as described in Chapter 5 were conducted to determine the influence of different ryegrass growth stages on glufosinate ammonium efficacy. Glasshouse experiments were conducted at Welgevallen experimental farm and the field experiments were conducted at Welgevallen, Roodebloem and Langgewens experimental farms. Growth stages of ryegrass were 2, 4, 6, 8 and 10 weeks. Applied dosage rates were 1.5, 3, 4.5, 6 and 7.5 L ha^{-1} for glasshouse experiments and 2.5, 5, 7.5 and 10 L ha^{-1} for field experiments. The findings of the study proved that growth stage of ryegrass has no influence on efficacy of glufosinate ammonium. However, differences in control were observed for different glufosinate ammonium dosage rates. The study also revealed better control of ryegrass in the glasshouse as compared to the field.

The trials described in Chapter 6 were conducted at Welgevallen experimental farm to investigate the efficacy of glufosinate ammonium with different added adjuvants on different aged ryegrass in both the glasshouse and the field. Three different growth stages of ryegrass (3, 6, and 9 weeks) were obtained by planting the ryegrass at 3-week intervals from the date the experiment was initiated, whilst young and mature growth stages were obtained in the field by spraying wild ryegrass at average leaf numbers of 6 and 15 leaves $plant^{-1}$, respectively. In the glasshouse, control of 6-week old ryegrass was more effictive regardless of the mixture applied. Glufosinate ammonium with the added adjuvant (Velocity®) controlled ryegrass more effectively than glufosinate ammonium applied alone or with another added adjuvant (Summit Super). Field experiment results showed that AMS added to glufosinate ammonium controlled young ryegrass better than glufosinate ammonium alone and with the adjuvant (Ballista®).

A glasshouse trial to compare efficacy of glufosinate ammonium on ryegrass and bahia grass species as influenced by temperature is described in Chapter 7. Applied dosage rates were 1.5, 3, 4.5, 6 and 7.5 L ha^{-1}. The glasshouse temperatures were set at 10/15, 15/20, 20/25 and 25/30 °C night/day temperatures. The findings of the study showed a similar trend in glufosinate ammonium control of both grasses; as temperature increased, control decreased. At 10/15 and 15/20 °C temperatures percentage control was significantly higher

than at 20/25 and 25/30 °C temperatures. Even though the trend was similar, mortality of ryegrass at high temperatures was more apparent as compared to bahia grass.

UITTREKSEL

Onkruiddoderweerstand is die vermoë van onkruide om te oorleef en voort te plant na toediening van die geregistreerde toedieningsdosis van ‘n onkruiddoder wat dodelik is vir die wilde ekotipe. Daar is wye kommer in landboukringe oor onkruide met hoë genetiese diversiteit wat weerstand teen chemiese onkruidbeheer ontwikkel het, raaigras (*Lolium* spp.) ingesluit. Raaigras het weerstand ontwikkel teen algemeen gebruikte onkruiddoders soos parakwat en glifosaat. Daar is ‘n geleentheid om glufosinaat ammonium te gebruik om probleme met onkruiddoderweerstand in raaigras te verlig. Die onkruiddoder het nie net ‘n unieke meganisme van werking nie maar daar is ook nog nie bewese weerstand teen dit in raaigras in Suid-Afrika gevind nie.

Daar is beperkings ten opsigte van toedieningstydperke met glufosinaat ammonium aangesien dit hoofsaaklik ‘n kontakonkruiddoder is. Meer spesifieke aanbevole toedieningsdosisse van die onkruiddoder kan dus ontwikkel word deur die bydrae van omgewingsfaktore en groeistadia tot die effektiwiteit van glufosinaat ammonium vas te stel. Die hoofdoelwit van die studie was om die mees effektiewe toedieningsdosis van glufosinaat ammonium vir die beheer van raaigras onder verskillende temperatuurregimes en groeistadia te bepaal. Studies oor die invloed van temperatuur op glufosinaat ammonium effektiwiteit is uitgevoer in Hoofstukke 3, 4 en 7. Die invloed van groeistadia op effektiwiteit van glufosinaat ammonium is ondersoek in Hoofstukke 5 en 6.

Glufosinaat ammonium toedieningsdosisse van 0, 1.5, 3, 4.5, 6 en 7.5 L ha^{-1} is gebruik vir ‘n temperatuurstudie in glashuise wat op 10/15, 15/20, 20/25 en 20/30 °C nag/dag temperature gestel was. In Hoofstuk 3 word die invloed van temperatuur op effektiwiteit van glufosinaat ammonium op jong en volwasse raaigrasplante beskryf. Die studie het gewys dat 3 L ha^{-1} glufosinaat ammonium sowat 95% beheer lewer op beide jong en volwasse raaigrasplante by ‘n temperatuur van 10/15 ºC terwyl die tendense by 15/20 en 20/25 nie duidelik was nie. By temperature van 25/30 ºC het swak beheer van raaigras voorgekom. Oor die algemeen is jong raaigras beter beheer as volwasse raaigras.

In Hoofstuk 4 word die effek van temperatuur op die effektiwiteit van glufosinaat ammonium met bygevoegde ammoniumsulfaat (AMS) op raaigras beskryf. Glufosinaat ammonium is toegedien teen toedieningdosisse van 1, 2 en 3 L ha^{-1} tesame met AMS byvoegings van 1, 2 en 3%. Glashuise is ingestel op 10/15, 15/20, 20/25 en 20/30 °C nag/dag temperature. Die resultate het getoon dat ‘n toedieningsdosis van 3 L ha^{-1} glufosinaat

ammonium met byvoeging van 2 en 3% AMS raaigras effektief beheer het. Daar was beter beheer van raaigras met byvoeging van al die AMS konsentrasies by laer temperature as by hoër temperature. ‘n Verhoging in AMS konsentrasie het gelei tot ‘n verhoging in raaigrasbeheer by laer temperature maar dit was nie sigbaar by hoër temperature nie.

Glashuis- en veldproewe soos beskryf in Hoofstuk 5 is uitgevoer om die invloed van verskillende raaigras groeistadia op glufosinaat ammonium effektiwiteit te bepaal. Glashuis eksperimente is uitgevoer op Welgevallen proefplaas en veldeksperimente is uitgevoer op die Welgevallen, Langgewens en Roodebloem proefplase. Groeistadia van die raaigras wat getoets is was 2, 4, 6, 8 en 10 weke oue plante. Toedieningsdosisse was 1.5, 3, 4.5, 6 en 7.5 L ha^{-1} vir die glashuisproewe en 2.5, 5, 7.5 en 10 L ha^{-1} vir die veldproewe. Resultate van hierdie proewe het gewys dat groeistadium van raaigras geen effek op die effektiwiteit van glufosinaat ammonium gehad het nie. Verskille in vlakke van beheer is egter waargeneem tussen verskillende glufosinaat ammonium toedieningsdosisse. Die studie het egter ook beter beheer van raaigras in glashuisproewe gewys as in veldproewe.

Die proewe wat in Hoofstuk 6 beskryf word het die invloed van verskillende bymiddels en raaigras groeistadia op die effektiwiteit van glufosinaat ammonium getoets in beide glashuis en veldproewe. Drie groeistadia van 3, 6 en 9 weke oue raaigrasplante is verkry deur die raaigrassaad met tussenposes van drie weke vanaf die aanvang van die proef in die glashuis te saai terwyl jong en meer volwasse plante in die veld gespuit is op twee verskillende stadia waarop die raaigras ongeveer 6 en 15 blare per plant onderskeidelik gehad het. In die glashuis was beheer van 6 weke oue plante baie beter ongeag die spuitmengsels wat toegedien is. Glufosinaat ammonium met Velocity® bygevoeg het raaigras beter beheer as glufosinaat ammonium sonder ‘n bymiddel of met byvoeging van Summit Super®. Die veldproef het getoon dat glufosinaat ammonium met bygevoegde AMS (2%) jong raaigras beter beheer het as glufosinaat ammonium alleen of met Ballista® bygevoeg.

‘n Glashuisproef wat die effektiwiteit van glufosinaat ammonium teen verskillende temperature op raaigras en bahiagras ondersoek het, word in Hoofstuk 7 beskryf. Toedieningsdosisse was 1.5, 3, 4.5, 6 en 7.5 L ha^{-1}. Die glashuistemperature was gestel op 10/15, 15/20, 20/25 en 25/30 °C nag/dag temperature. Die resultate wys soortgelyke neigings in terme van glufosinaat ammonium beheer vir beide spesies naamlik dat die beheer afneem soos temperature toeneem. Teen 10/15 en 15/20 ºC was beheer betekenisvol hoër as by 20/25

en 25/30 ºC. Alhoewel die neigings eenders was, was beheer van raaigras by hoër temperature swakker as beheer van bahiagras.

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TABLE OF CONTENTS

PREFACE

This dissertation consists of eight chapters. Chapter 1 gives a brief background information of the study, justifies the reason for conducting the study and outlines the objectives of the study. A review of literature that is relevant to the study is given in Chapter 2. Chapters 3 to 7 consists of experiments presented in complete paper format with an introduction, specific objectives, hypotheses, materials, method, results, discussion and conclusion. Considering the outline here, the format and wording of a greater part of the methodology section will be duplicated for Chapters 3, 4 and 7. This will be also noticed for Chapter 5 and 6 due to their similarities in methods. Chapter 8 summarises the findings from the experiments as well as provide recommendations. All the references cited in the study are found in the reference list at the end of each chapter. An appendices section containing outputs of statistical analyses of data presented in the paper is placed at the end of this dissertation.

LIST OF ACRONYMNS

AMS	ammonium sulphate
ARC	Agricultural Research Council
COC	crop oil concentrates
et al.	refers to a number of people
IWM	integrated weed management
L ha^{-1}	litres per hectare
NH_4^+	ammonium ion
NIS	non-ionic surfactants
LSD	Least significant difference
ANOVA	Analysis of variance
cv	commercial variety
spp	refers to all species in a given genus

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CHAPTER 1

THE EFFICACY OF GLUFOSINATE AMMONIUM ON RYEGRASS AS INFLUENCED BY DIFFERENT PLANT GROWTH STAGES AND DIFFERENT TEMPERATURES

1. GENERAL INTRODUCTION

1.1 Background

Occurrence of herbicide resistant weeds is common in South Africa, particularly in the western Cape (ARC Report 2013/2014.). The most troublesome weed is ryegrass (*Lolium* spp.). The ARC annual report also noted that billions of rands have already been spent towards its control. The weed's persistence is mainly due to its ability to undergo natural mutation, and hence it has the propensity to evolve resistance to a variety of herbicides. Weed resistance is defined as the ability of weeds to survive and reproduce following exposure to the recommended dosage rate of a herbicide that is lethal to its wild type (Vencill et al. 2010).

A number of factors result in the development of herbicide resistance in weeds, and these include continuous application of the same herbicide, the nature of the herbicide being applied, simple cropping systems that favor a dominant weed species, residual soil seed bank of weeds and inherent factors in the plant population (Powles et al. 1996; Beckie 2006). Continuous application of the same herbicide or herbicides with the same mode of action will create selection pressure, thus more resistant weeds contribute a disproportionate number of progeny to the next generation (Tharayil-Santhakumar 2003)

The widespread occurrence of resistant weeds has led to a dire need for integrated weed management (IWM) strategies (Llewellyn et al. 2004). Integrated weed management is an approach that uses several techniques such as biological, chemical, cultural and physical weed control. Llewellyn et al. (2004) also noted that adoption of IWM has been negative because farmers prefer weed strategies that are noticeably profitable in a short space of time. Adoption of eco-farming and conservation agriculture has also led to a high increase of herbicide use in weed control (Ahmadi et al. 1980). These practices encourage farmers to produce crops with little or no tillage hence they opt for chemical use. Herbicide use is therefore a prominent weed management strategy that is being exploited due to its immediate high profit returns.

A study to determine the most effective method of ryegrass control was conducted by Pline et al. (2000) who concluded that no IWM practice or pre-emergent herbicide offered the

reliability that post-emergence herbicides offered. Introduction of post-emergence weed management with non-selective broad spectrum herbicides has been a great achievement in agriculture after the introduction of herbicide-resistant crops (Everman 2008). Herbicide resistant crops have made weed management much easier despite the fact that only a few transgenic herbicide traits are commercially available after three decades of research (Green and Owen 2011). According to Green and Owen (2011) the commonly used post-emergence non-selective herbicides are glyphosate and paraquat due to the high popularity of transgenic crops that are resistant to them.

Adoption of herbicide-resistant crops, particularly the glyphosate-resistant ones, enabled effective, easy, economic, safe and novel management of weeds, to such an extent that farmers extensively used glyphosate as a major weed treatment (Green and Owen 2011). Gradually weeds have developed resistance to glyphosate. Boutsalis et al. (2009) lamented that no new modes of action have been marketed since the 1980s after the evolvement of annual ryegrass resistance to most of the post-emergence herbicides. According to Pieterse (2010) weeds, including ryegrass have been reported to have developed resistance to glyphosate but no cases of herbicide resistance to glufosinate ammonium have yet been confirmed in South Africa. Therefore, glufosinate ammonium can be a viable option in the control of weeds, ryegrass included, provided that transgenic glufosinate ammonium crops are introduced in South Africa.

Coetzer and Al-Khatib (2001) describes organophosphorus glufosinate ammonium as an active ingredient salt which is available in liquid formulation under the product name Basta® and other commercially available herbicides named Rely®, Finale®, Challenge®, LibertyLink® and Pestanal®. Glufosinate ammonium inhibits the glutamine synthetase enzyme which is responsible for amino acid glutamine production, and hence, results in ammonia toxicity which eventually restricts plant photosynthesis (Everman 2008). It was reported that phytotoxity symptoms of glufosinate ammonium develop rapidly on weeds when compared to glyphosate, however, when applied at similar rates glyphosate action exceeds that of glufosinate (Pline et al. 2000). In contrast, glufosinate ammonium has been noted to be more effective than glyphosate in another study but there was variation of tolerance and sensitivity amongst the weed species (Pline 1999a).

Everman et al. (2009) noted glufosinate ammonium requires complete coverage in controlling weeds since it is a contact herbicide. Many studies have shown different

sensitivities and tolerance of weeds to glufosinate ammonium (Pline et al. 1999b; Everman et al. 2009). Quite a number of factors contribute to the different tolerance levels, including: temperature, humidity, plant growth stage, application rate, application timing, weed species and plant physiological factors such as absorption and translocation (Everman 2008). This study serves therefore to investigate the effective dosage rates of glufosinate ammonium in the control of ryegrass weed under different temperatures and at different plant growth stages. Efficacy of glufosinate ammonium under specific conditions was also investigated with added adjuvants.

1.2 Study objectives

The principle objective of the study was to determine the effective dosage rate of glufosinate ammonium in the control of ryegrass weed under different temperature regimes and ryegrass growth stages. The overall study question was; at what glufosinate ammonium dosage rates can ryegrass weeds be controlled effectively at different temperatures and ryegrass growth stages? The specific objectives were as follows;

A. To determine the effect of temperature on the efficacy of glufosinate ammonium in a glasshouse
B. To determine the effective dosage rate of glufosinate ammonium under different temperatures using ammonium sulphate as an adjuvant
C. To determine an effective dosage rate of glufosinate ammonium for ryegrass at different growth stages under glasshouse and field conditions
D. To determine the effective dosage rate of glufosinate ammonium under different growth stages of ryegrass using ammonium sulphate and vegetable oil as adjuvants
E. To compare efficacy of glufosinate ammonium at low and high temperatures on temperate and tropical grass species

The overall null hypothesis of the study is as follows:

H_0: temperature and growth stage has no effect on the efficacy of glufosinate ammonium in controlling ryegrass weeds.

The null hypotheses for the specific objectives are as follows:

A. H_0: temperature and ryegrass growth stage have no effect on the efficacy of glufosinate ammonium in controlling ryegrass weeds.

B. H_0: temperature has no effect on the efficacy of glufosinate ammonium with adjuvant ammonium sulphate in controlling ryegrass weeds.
C. H_0: ryegrass growth stage has no effect on the efficacy of glufosinate ammonium in controlling ryegrass weeds.
D. H_0: ryegrass growth stage has no effect on the efficacy of glufosinate ammonium which has ammonium sulphate or vegetable oil as adjuvants
E. H_0: there is no difference in the trend and efficacy of glufosinate ammonium between tropical and temperate grass species

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CHAPTER 2

THE EFFICACY OF GLUFOSINATE AMMONIUM ON RYEGRASS AS INFLUENCED BY DIFFERENT PLANT GROWTH STAGES AND DIFFERENT TEMPERATURES

LITERATURE REVIEW

2.1 Ryegrass proliferation in South Africa

There is a widespread concern in agriculture about weeds with high genetic diversity that have developed resistance to weed control (Powles et al. 1998; Martin et al. 2001; Duke and Cerdeira 2005; Renton et al. 2014). A morphological study done by Ferreira et al. (2015) in the western Cape on identification of genetic variation of ryegrass (*Lolium* species), observed that rigid ryegrass had the highest occurrence in populations of 50%, 48% of the populations was classified as the hybrid and perennial ryegrass occurred in 2% of the total number of populations. Classification of the ryegrass samples was done through studying ryegrass inflorescence, ryegrass seed morphology and comparing with characteristics of the existing herbarium specimens.

Proliferation of weedy ryegrass has been observed to cause more negative impacts than other weeds such as wild oats (Todd 2008). Ryegrass species' inherent genetic traits and its biological advantage over other crops and plants give them notable plasticity (Peppas et al. 2006; Ferreira et al. 2015). Ryegrass was reported to establish itself earlier than all species, which explains its significant competitive advantages. Todd (2008) also highlighted that ryegrass can establish extensive seed banks and is less preferred by livestock, hence, it develops dense stands which not only compete for light but also creates high biomass that implies heavy mulching which smothers emerging plants.

Another added advantage to the plasticity of ryegrass is brought about by its climatic requirements. Andy (2007) highlights that annual ryegrass is a temperate pasture crop that can establish its seed in cool soil, and is frost tolerant. Furthermore, the western Cape predominantly receives its rainfall in winter, consequently providing favourable conditions for ryegrass. Winter crops are seeded from late April through June, therefore, they are placed in cold soils which increases their susceptibility to weed competition because the crops have slow early growth (Sutherland n.d.). He also noted that the grass weeds do not only compete with crops but also harbours cereal root diseases.

Pest control in winter cropping systems deserves much attention to ensure that high yields and good quality crops are produced. Integrated weed management strategies include crop rotations, managing the seed bank, crop competition, cultivations, weather, crop, herbicide choice, application technique and timing (Cook et al. 2010). Andy (2007) reported that mechanical disking is the most effective way to kill ryegrass. Use of contact herbicides is another effective way of eradicating ryegrass. However, cases of resistance of ryegrass to certain herbicides have been reported and this is mainly due to misuse of these herbicides (Andy 2007).

In Australia, ryegrass has been reported to have developed cross-resistance to herbicides such as acetyl coenzyme A carboxy-lase (ACCase)-inhibiting herbicide (diclofop-methyl) and acetolactate synthase (ALS)-inhibiting herbicide (chlorsulfuron) (Han et al. 2015). It has become a challenging weed because it has shown great capability to evolve herbicide resistance to various herbicide modes of action (Stanton et al. 1998). Ryegrass has developed resistance to paraquat and glyphosate through the process of mutation and selection. The grass is an out-crossing open pollinated variety and has great capability of undergoing hybridization. As a result, ryegrass has high levels of genetic diversity that explain its survival, regeneration and adaption traits in multiple ecosystems (Matzrafi et al. 2014; Ferreira et al. 2015).

2.2 Herbicide resistance in weeds

Martin et al.(2001) defines herbicide resistance as an inheritable ability of species to survive a dose of herbicide lethal to its wild type. Requirements for the evolution of resistant species are occurrences of a heritable variation of trait and natural selection (Pieterse 2010; Ferreira et al. 2015). Resistant individuals that are present in the population will initially be a small fraction of the population but due to disproportionate high seed production, resistance traits will be inherited to the next generation, and hence, development of large fractions of herbicide-resistant weeds is observed. **Figure 2.1** illustrates how selection of herbicide-resistant weeds occurs. The frequency of mutants and characteristics of applied herbicides determine the rate of development of herbicide-resistant weeds (Pieterse 2010; Vencill et al. 2010).

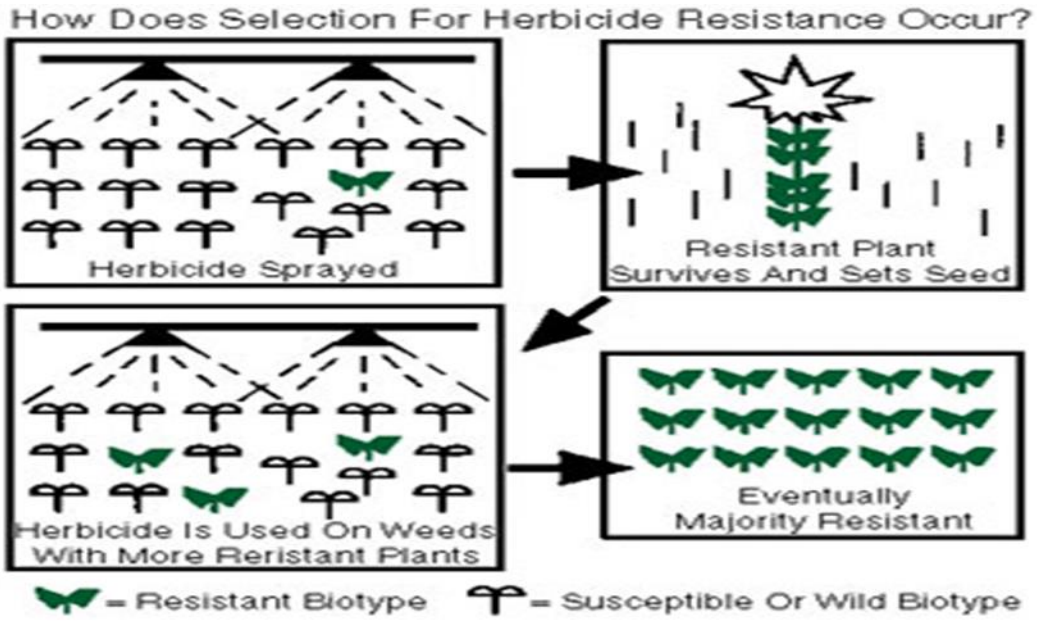

Figure 2.1: Selection for herbicide resistance –Adapted from Gunsolus (2008)

2.2.1 Target site herbicide resistance mechanisms

Resistance mechanisms can be categorized into two groups namely target site resistance and non-target site resistance. Target site resistance involves mechanisms that resist herbicide action on the target. This mechanism includes altered site of action resistance and overproduction of the target site.

a. Altered site resistance

Altered site of action resistance is chiefly associated with mutation in the gene coding for a protein which results in changes of a protein structure (Powles et al. 1996). Herbicides usually work by disrupting an enzyme or protein that plays a key role in plant biochemical processes, thus when there is mutation, the physical changes impair the ability of herbicides to attach to the binding site. Beckie (2006) noted that target site resistance accounts for the majority of herbicide-resistant biotypes. He also records that this mechanism results in rapid evolution of weed resistance to herbicides that are applied at registered rates.

b. Enzyme overproduction

The plant might produce large quantities of target site enzymes, hence, causing increased numbers of the target sites so that the herbicide applied will not be able to inactivate the

entire number of enzymes produced (Tharayil-Santhakumar 2003). Surviving enzymes will continue with normal plant metabolic activities.

2.2.2 Non-target site herbicide resistance mechanisms

Non-target site resistance is a combination of mechanisms in plants that result in limiting the amount of herbicide that reaches the target site. The mechanisms involved include decreasing absorption of herbicides, decreasing translocation, redirecting herbicides into many locations and enhanced metabolism (Tharayil-Santhakumar 2003; Powles and Yu 2010).

a. Enhanced herbicide metabolism

Enhanced herbicide metabolism is the ability of a plant to metabolically degrade herbicide molecules to form a substance which is no longer toxic. According to Powles and Yu (2010), an example of enhanced metabolism is observed in crops like maize and wheat and the process involves an increase in cytochrome P450 mono-oxygenases that results in herbicide conversion by hydroxylation or dealkylation. Metabolized herbicide by P450s will reduce their phytotoxicity and they are further converted to glucose and consequently transported into the plant system.

b. Reduced absorption and translocation

Reported resistance of some *Lolium* species to glyphosate involves its restricted translocation and absorption (Powles and Yu 2010). According to Tharayil-Santhakumar (2003), the apoplastic and symplastic systems of resistant plants is modified in such a way that absorption and translocation is limited or restricted.

c. Redirecting herbicides into other locations before reaching target site

Some plants are capable of sequestering herbicides into compartments such as vacuoles before reaching the target site. This may result in immobilization of some herbicides (Tharayil-Santhakumar 2003). Although the mechanism is not yet understood, it is speculated that the vacuole membrane pumps glyphosate into vacuoles where it is sequestrated.

2.3 Management of herbicide-resistant weeds

2.3.1 Non-chemical management

Weed management does not necessarily mean chemical control (Cook et al. 2010). Other methods can be exercised in trying to ensure effective weed control. These include mechanical methods such as tillage, cutting, mowing, crushing, digging and stump excavation (Cook et al. 2010). Ryegrass is effectively controlled by mechanically disking (Andy 2007). Other multiple strategies that can be implemented include practicing crop rotations, practicing mixed farming systems, using crop canopy and diversifying crop sequence. Biological control of weeds is another non-chemical strategy that is useful in weed management. It basically involves introductions of non-native species into a community and also manipulation of the indigenous populations (Cook et al. 2010). However, biological methods have been reported to produce superlative results only after a long period of time after they are initiated, hence, these methods are more suited to perennial and biennial crops (Llewellyn et al. 2004).

2.3.2 Chemical management

A relief from strenuous mechanical weed management caused by chemical management led to increased dependence on herbicides, hence, crop rotations and alternative weed control techniques were abandoned in many instances (Pieterse 2010). Herbicides are very effective tools for weed management since they are cost effective, however, their overuse result in development of resistance of weeds (Martin et al. 2001). Martin et al. (2001), therefore, clarifies that it is important to adopt integrated weed management strategies since weeds adapt poorly to changing management systems.

In cases where herbicides are used for weed management it is important to pre-plan the weed control strategy. A pre-planned control strategy should include scouting the field before and after application of herbicides, use of herbicides when necessary, use recommended dosage rates of herbicide and rotation of herbicides (Martin et al. 2001). Scouting the field helps in detecting weed escapes and shifts therefore available control methods to avoid seed disposition are implemented if there are any detections of potential resistant weeds.

Introduction of post-emergence weed management by non-selective broad spectrum herbicides has been a great achievement in agriculture especially after the introduction of herbicide-resistant crops (Petersen and Hurle 2000). Commonly used herbicides by farmers in the western Cape are paraquat and glyphosate. Unfortunately, ryegrass has developed

resistance to these herbicides. It is therefore important to introduce more effective herbicides to farmers since they have been reported to mostly prefer chemical control (Llewellyn et al. 2004). There is an opportunity of using glufosinate ammonium herbicide in controlling ryegrass since no resistance to it has yet been proven for this herbicide (Pieterse 2010).

2.3 Control of ryegrass with glufosinate ammonium

Glufosinate ammonium is a non-selective post-emergence contact herbicide which requires thorough or complete coverage to ensure good control (Li et al. 2014). It is a phosphorus containing amino acid with chemical name 2-amino-4-(hydroxymethylphoshinyl)butanoic acid ; (Martinson et al. 2002; Everman 2008; Everman et al. 2009). Glufosinate ammonium, also known as phosphinothricin is a non-volatile salt, soluble in polar solvents and water and insoluble in non-polar organic solvents. The structural formula of glufosinate ammonium is shown in Figure 2.2.

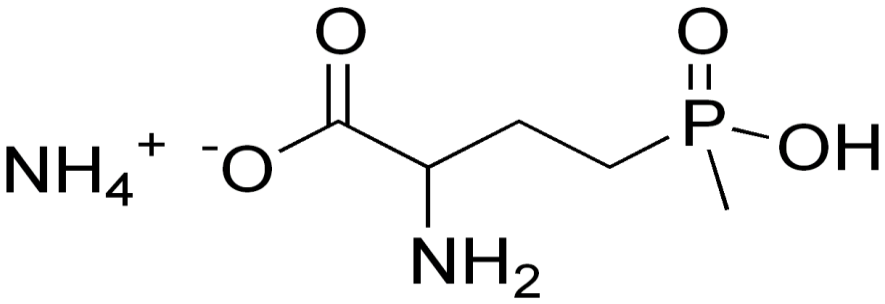

Figure 2.2: Structural formula of glufosinate ammonium (Faber et al. 1998)

Glufosinate ammonium is mainly used as a desiccant on weeds, to facilitate harvest and also for selective use in genetically modified glufosinate-tolerant crops. Glufosinate ammonium controls several annual and perennial grasses and broadleaf weeds (Coetzer and Al-Khatib 2001). According to Everman (2008), glufosinate ammonium is readily degraded by microorganisms, hence, it is found in the upper layer of the soil because less is available for leaching after the degradation process. It has no residual activity. Since glufosinate ammonium is a contact herbicide, damage is restricted to those parts of the plant that have been in contact with the spray (Pline et al. 1999).

2.4.1 Glufosinate ammonium mode of action

Glufosinate ammonium inhibits the glutamine synthetase enzyme which is responsible for amino acid glutamine production and ammonia detoxification (Everman 2008). The inhibition results in decreased glutamine and increased ammonia levels in the plant's tissues (Pline et al. 2000). Photosynthesis is thereby stopped and plants die within a few days after

application of the herbicide due to necrosis which is a result of damage in the chloroplast structures (Everman 2008). Everman (2008) also noted that the role of accumulation of ammonia in phytotoxicity is not clear, however, the symptoms include membrane disruption and inhibition of photosynthesis which is eventually followed by death of the plant.

According to Pline et al. (1999a) glufosinate ammonium mode of action involves three steps: (i) inhibition of protein biosynthesis occurs due to lack of glutamine production (ii) a toxic accumulation of glyoxylate which inhibits RuBP-carboxylate and carbon dioxide fixation and (iii) an interruption of photorespiration that results from deficiency of intermediates of the Calvin cycle. Petersen and Hurle (2000) stated that the process occurs in leaf tissue due to photorespiration and nitrogen assimilation, hence, ammonia accumulation after glufosinate ammonium treatment only occurs in the light. Timely application is recommended when applying glufosinate ammonium to ensure effective weed control.

2.4.2 Absorption of glufosinate ammonium

Absorption of glufosinate ammonium takes place mostly through foliage and little or none in the roots under field conditions because of rapid microbial breakdown. The herbicide's uptake is mainly influenced by factors influencing the activity of glufosinate ammonium and these include relative humidity, temperature and morphological and biochemical plant factors (Petersen and Hurle 2000). Efforts have been made to determine adjuvants that can increase glufosinate ammonium uptake. The use of additives like crop oils and a large range of wetting agents have not assisted in absorption of glufosinate ammonium (Pratt et al. 2003). Petersen and Hurle (2000), however, noted that the adjuvant ammonium sulphate increased glufosinate ammonium uptake.

2.4.3 Translocation of glufosinate ammonium

Glufosinate ammonium has been shown to have little translocation out of treated leaves due to its contact phytotoxicity (Pline et al. 1999b). Petersen and Hurle (2000) noted that relative humidity not only affects the herbicide's uptake but also its transpirational flow. High flow rates of water in the xylem will cause the herbicide concentration to be diluted, and hence, translocation in the treated leaves is reduced. Pline et al. (1999a) also noted that in some plants it has been observed that glufosinate ammonium is mobile in grasses such as *Setaria vidiris* L., *S. faberi* and *Hordeum vulgare* L. In such plants it was noted that it is more phloem mobile than xylem mobile. A study done by Everman et al. (2009) concluded that more than

90% of glufosinate ammonium herbicide remained in the treated leaves, except in the case of glufosinate-resistant maize which displayed significant mobility of the herbicide to the roots.

2.4.4 Role of adjuvants

Spray applications of aqueous solutions, suspensions or emulsions are used to obtain the evenness of herbicides when spraying and also to avoid drifting (Van Acker 2009). These substances are called adjuvants. Adjuvants are substances formulated in herbicides or added to the spray tanks in order to modify the activity or the application characteristic of herbicides (Felix et al. 2012). Glufosinate ammonium has been reported to be effective with its formulated wetting agent even though many additives have been tested with it. Despite the fact that it works best without additives, it has been noted that ammonium sulphate (AMS) improves uptake and efficacy of glufosinate ammonium in some situations (Bayer crop science n.d.). **Table 2.1** below gives a summary of herbicide adjuvants and their functions.

Table 2.1: Summary of herbicide adjuvants (Curran et al. 1999)

Spray adjuvant	Examples	Function
Surfactants	1.Non-ionic surfactants (NIS) 2.Silicon surfactants	Spreader-wetter-penetrant
Crop oil concentrates (COC)	1.Petroleum oil concentrate 2.Methylated oil concentrate	Penetrant spreader Humectant
NH_4 adjuvants	1.Ammonium sulphate (AMS) 2.Urea ammonium nitrate	Spray solution buffer Increase herbicide activity
Compatibility agents		Aiding mixing with liquid fertilizer
Drift retardants		Reduce spray drift

Pratt et al. (2009) postulated that the same mechanisms through which ammonium sulphate increases efficacy of glyphosate apply to glufosinate ammonium, since the two herbicides have similar structures. The role of AMS in glyphosate activity is achieved when the NH_4^+ cation from AMS binds to the glyphosate molecule, and the SO_4^- anion binds to cations such as Mg^{2+}. This prevention is necessary since glyphosate readily reacts with cations in water, such as calcium and magnesium, to form a less soluble salt that is poorly absorbed by plants (Pratt et al. 2003). In contrast, the NH^4-glyphosate molecules are readily absorbed into the leaves.

2.5 Influence of environment and plant factors on glufosinate ammonium efficacy

2.5.1 Influence of environmental factors

Coetzer et al. (2001) reported that environmental conditions influence efficacy of post-emergence herbicides directly before, during and after application. Therefore, it is of paramount importance to understand how the environment influences the efficacy of herbicides so as to develop effective management of weeds (Kumaratilake and Preston 2005). In terms of the role of rain, Everman (2008) stated that glufosinate ammonium has a rain-free period of four hours for most weed species. It is advised that the herbicide is applied at the time of the day when the wind speed is low to avoid herbicide drift (Mohr et al. 2007). Herbicide drift results in poor control of the target species and it may also damage non-target species.

Relative humidity, light intensity and temperature influence glufosinate ammonium efficacy (Coetzer et al. 2001). Coetzer et al. (2001) postulates that high relative humidity enhances absorption and translocation by increasing cuticle hydration, as well as prolonging the drying time of droplets. Low relative humidity decreases water content of the cuticle and consequently the hydrophobic character of cuticle increases, hence, penetration of water soluble compounds like glufosinate ammonium is decreased (Petersen and Hurle 2000). Petersen and Hurle (2000) observed that humidity and light intensity had a greater influence on glufosinate ammonium efficacy than temperature.

In a study done by Archambault et al. (2001), it was observed that the herbicide's action was effective at high temperatures immediately after its application but prolonged exposure to high temperature decreased its efficacy. Coetzer et al. (2001) reported that warm temperatures generally increase the toxicity of glufosinate ammonium due to increased absorption and affinity of herbicide the to the binding site. A study conducted to observe the effect of temperature on glufosinate ammonium efficacy in wild radish (*Raphanus sativus*) concluded that glufosinate ammonium efficacy was greater under higher temperatures of 15/20 and 20/25 °C as compared to 5/10 °C (Kumaratilake and Preston 2005).

Effects of the environment on plant factors can also influence herbicidal activity. For instance, low humidity and air temperatures influence the cuticle. Low humidity has been reported to dehydrate the cuticle, hence, reducing absorption of water soluble herbicides like glufosinate ammonium (Steckel et al. 1997). Steckel et al. (1997) also highlighted that air temperature changes the permeability of both the cutin matrix and the soluble membranes in

some species. However, plant response to herbicides has been reported to vary among species, since high air temperatures tend to speed up absorption and translocation, but on the other hand, it might encourage rapid metabolism which reduces herbicide activity (Smeda and Putnam 2010; Varanasi et al. 2015).

2.5.2 Influence of plant factors

Knowing the reproductive biology and patterns of growth of a weed is important in increasing efficacy of herbicides (Norsworthy et al. 2012). Several plant factors influence the efficacy of herbicides even when the environmental conditions are optimized. Plant factors have an effect on retention, uptake, translocation and metabolism of glufosinate ammonium (Petersen and Hurle 2000). These factors include plant size, thickness of the cuticle, leaf angle, leaf area and diurnal leaf movements.

Plant growth and physiology are influenced by the environment. Light has been reported to positively affect leaf angle, leaf movement, stomatal conductance and leaf cuticle (Mohr et al. 2007). Mohr et al. (2007) also highlighted that reduced leaf area due to the angular orientation of the leaf can result in decreased efficacy of herbicides. The opening of stomata, thinner leaves with greater specific area and higher plant branching increases surface coverage, thus improving the penetration of foliar applied herbicides (Varanasi et al. 2015).

Cook et al. (2010) stated that young weeds are generally easier to control and the ease of controlling most of the weeds declines as weeds grow. Young plants that are actively growing, have thinner leaves compared to mature plants and the cuticle is much more permeable improving efficacy of foliar herbicides (Steckel et al. 1997). As a result, glufosinate ammonium, a water-soluble herbicide is more effective in penetrating the cuticle of smaller weeds than mature weeds. However, in some species, actively growing plants have increased metabolism of herbicide-degrading enzymes, hence, herbicide activity is impaired and consequently the young weeds are difficult to control (Varanasi et al. 2015).

2.6 Opportunities and constraints of glufosinate ammonium

According to Green and Owen (2011), glufosinate ammonium is a very effective herbicide which is fast acting and controls broadleaved weeds and grasses. Green and Owen (2011) stated that there is no weed resistance to glufosinate ammonium that has been documented yet but this is contrary to other recent findings which have proved resistance of goosegrass (*Eleusine coracana*) and Italian ryegrass (*Lolium perenne*) to glufosinate ammonium (Jalaludin et al. 2010; Seng et al. 2010; Avila-Garcia and Mallory-Smith 2011).

In weed management systems, which rely solely on post-emergence weed control, crop yield losses due to early season weed interference are common (Everman 2008). However, Everman (2008) also noted that introduction of glufosinate-resistant crops has provided a perfect management tool for farmers employing such systems. Yield loss with herbicide-resistant crops is not significant hence post-emergence option control has been totally adopted in weed management for cotton, canola, and maize among others. The use of such crops is a possibility for herbicide rotations and is therefore a tool for improved weed management that is going to reduce chances of herbicide resistance (Palou et al. 2008). A challenge, however, as stated by Palou et al. (2008) will be when glufosinate ammonium is used continuously by farmers. Flora invasions and weed resistance might greatly increase under such conditions.

Glufosinate ammonium is readily degraded by microorganisms, hence, it has no residual activity and contamination of groundwater is unlikely (Everman 2008). Everman (2008) also reported that in non-sterile environments, degradation of glufosinate ammonium takes about 1-10 days in loam soils, about 4 days in forest soils and 15-25 days in clay and clay loam soils. Therefore, replanting and rotation concerns are generally minimal under field conditions. The herbicide degradation process involves the formation of 3-(hydroxymethylphosphinyl) propionic acid as a degradation product (Jansen et al. 2000). The study observed that the degradation products underwent slow degradation with release of carbon dioxide and also incorporation into soil microbial biomass, as well as fulvic, humic and humin soil fractions that are necessary for improving soil fertility.

Glufosinate ammonium effectively controls a wide variety of weeds, thus obviating the need for tillage. No-tillage or reduced tillage is a chief component of Conservation Agriculture systems, and aimed at not only improving soil fertility but also reducing manpower and labour, hence, it is economically acceptable. However, glufosinate ammonium is used at higher rates and has been reported to be more expensive than other non-selective herbicide like glyphosate (Green and Owen 2011). Green and Owen (2011) also reported that there are restrictive application timings with glufosinate ammonium in terms of growth stage of weeds, since it is a contact herbicide and requires significant translocation to react effectively. More defined recommended dosages of herbicides can be developed by determining the contribution of environmental factors and growth stage of weeds to efficacy of herbicides (Smeda and Putnam 2010).

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CHAPTER 3

EFFICACY OF GLUFOSINATE AMMONIUM ON RYEGRASS AS INFLUENCED BY DIFFERENT TEMPERATURES AND PLANT GROWTH STAGES

ABSTRACT

Glasshouse experiments were conducted at Welgevallen experimental farm to evaluate the effect of temperature and ryegrass growth stage on the efficacy of glufosinate ammonium. The experiment was done on mature and young commercial ryegrass in which mature ryegrass was sprayed 6 weeks after planting while young ryegrass was sprayed 3 weeks after planting. Applied glufosinate ammonium dosage rates were 0, 1.5, 3, 4.5, 6 and 7.5 L ha^{-1}. Glasshouses were set at 10/15, 15/20, 20/25 and 25/30 °C night/day temperatures. The findings of the study indicated that for 15/20, 20/25 and 25/30 °C temperatures, the most effective dosage rate in controlling young ryegrass was 4.5 L ha^{-1} while 6 L ha^{-1} dosage rate was effective for mature ryegrass. However, 3 and 4.5 L ha^{-1} glufosinate ammonium dosage rates controlled young and mature ryegrass at 10/15 °C, respectively. At higher dosage rates of 6 and 7.5 L ha^{-1}, temperature had no significant effect on glufosinate ammonium efficacy. There was a significant effect of ryegrass growth stage on efficacy of glufosinate ammonium. When sprayed at the same rate under the same temperature, control of young ryegrass was 30% better than that of mature ryegrass.

Keywords: dosage rate, efficacy, glufosinate ammonium, growth stage, ryegrass, temperature.

3.1 INTRODUCTION

Herbicide resistance in ryegrass (*Lolium* spp) has resulted in severe yield reductions of field crops in the western Cape (Molefe 2015). Herbicide resistance has also complicated control of ryegrass with most of the commonly used herbicides that include paraquat and glyphosate (Eksteen et al. 2005). There is a possibility of using other alternatives such as glufosinate ammonium. However, inconsistencies in the response of different weed species to glufosinate ammonium under different temperatures have been reported (Coetzer and Al-Khatib 2001; Kumaratilake 2002; Kumaratilake and Preston 2005). Temperature affects both plant metabolism and herbicide efficacy. Mahan et al. (2006) noted that thermal dependency of herbicide action limits their activity and this can be explained with respect to reaction rates and kinetic constants.

According to Mahan et al. (2006), this knowledge was useful in determining optimal weed control temperatures. Penner (2015) noted that within a temperature range of 10 ºC to 30 ºC, an increase in temperature will enhance the phytotoxity of herbicides. In a study done by Smeda and Putnam (2010), control of green foxtail decreased as temperature increased from 18 to 30 °C while no temperature effect was shown on Japanese millet. Penner (2015) attributes enhanced efficacy of herbicides at higher temperatures to the increased herbicide uptake and translocation in plants but decreased efficacy can be attributed to volatilization of the herbicide at higher temperatures. Contrasting reactions might be due to differences in metabolism of plants that are grown under different temperatures (Kumaratilake and Preston 2005).

In a study conducted to observe the effect of temperature on glufosinate ammonium efficacy in wild radish, it was concluded that glufosinate ammonium efficacy was greater under higher temperatures (Kumaratilake and Preston 2005). At the active dosage rate of 600 g ha^{-1} glufosinate ammonium, 100% mortality of wild radish was observed at 20/25 °C, 70% mortality was observed at 15/20 °C and 20% mortality was observed at 5/10 °C. The study concluded that absorption of glufosinate ammonium is not greatly influenced by temperature but in contrast translocation is highly depended on temperature (Kumaratilake and Preston 2005). In a similar experiment, it was observed that rapid translocation of glufosinate ammonium at high temperature was also dependent on relative humidity. Low relative humidity decreases the efficacy of glufosinate ammonium. This is because high relative humidity results in slow herbicide evaporation and drift enhancing absorption and translocation (Coetzer et al. 2001; Jamal 2011).

Contact herbicides like glufosinate ammonium translocate very little or not at all from the point of entry, however, under favorable conditions, they can be subjected to limited movement (Rao 2000; Kumaratilake and Preston 2005; Everman et al. 2009). Among many factors that affect translocation of herbicides, temperature and plant maturity have considerable effects on the efficacy of herbicides. Generally, herbicides tend to translocate faster in younger plants than in older plants because uptake is faster in actively growing meristematic tissue (Rao 2000). Rao (2000) also noted that increased translocation at higher temperatures is due to the increased phloem activity.

The overall objective of this study was to determine the most effective dosage rate of glufosinate ammonium on ryegrass at different temperatures and ryegrass growth stages.

Specific objectives were, (i) to observe the trend in which temperature influences glufosinate ammonium efficacy, (ii) to determine the effective dosage of glufosinate ammonium at different temperatures, and (iii) to assess response of ryegrass to glufosinate ammonium at two different growth stages. The overall null hypotheses tested was; temperature and ryegrass growth stage has no influence on efficacy of glufosinate ammonium.

3.2 MATERIALS AND METHOD

3.2.1 Experimental site

The experiment was conducted in a glasshouse at the Stellenbosch University Welgevallen experimental farm. The site is located at 33° 56´33" S and 18° 51´56" E at an altitude of 136 m above sea level.

3.2.2 Treatments and experimental design

Commercial ryegrass (*Lolium multiflorum* cv Energa) seed were used in this experiment due to their ease of germination. A randomized complete block design arranged as a 2×4×6 factorial with 5 replications was used for the experiment. The experimental factors were ryegrass at two levels (mature and young), temperature at four levels (10/15, 15/20, 20/25 and 25/30 ºC night/day) and glufosinate ammonium dosage rates at six levels (0, 1.5, 3, 4.5, 6 and 7 L ha^{-1}).

3.2.3 Trial establishment and management

Planting

Seeds were first germinated in petri dishes in an incubator at 20 ºC under light conditions to ensure higher percentage germination and the resulting seedlings were transplanted into 8 x 8 cm square plastic pots after 2 weeks.

Weed, pest and disease control

Additional weeds were removed by hand. No pests and diseases were experienced in the glasshouse.

Irrigation

An automated irrigation system was used to water the plants. The plants were irrigated at 8:00 am, 12:00 pm, 2:00 pm and 4:00 pm. The quantity of water per irrigation was adjusted depending on the plant growth stage to compensate for water loss.

Fertilization

A nutrient solution was used to fertilize ryegrass plants during the study. The composition is shown **Table 3.1**.

Table 3.1: Composition of the nutrient solution used to fertilize the plants growing in pots.

EC = 2.0			
Element (Macro)	Concentration mg L^{-1}	Fertilizer	Concentration g $1000L^{-1}$
K^+	237.7	$KN0_3$	303
Ca^{++}	180	K_2S0_4	261
Mg^{++}	48.6	$Ca(N0_3)_2.2H_20$	900
$N0_3^-$	661.33	$MgS0_4.7H_20$	492
H_2P0_4	116.4	KH_2P0_4	136
$S0_4$	390.4		
(Micro)	mg L^{-1}		
Fe:	0.85	Libfer (Fe EDTA)	6.54
Mn	0.55	Manganese sulphate	2.23
Zn	0.30	Zinc sulphate	1.33
B	0.30	Solubor	1.46
Cu	0.05	Copper Sulphate	0.20
Mo	0.02	Sodium Molibdate	0.13

Herbicide application

The young plants were sprayed at 3 weeks after transplanting whereas mature plants were sprayed at 6 weeks after transplanting. The herbicide was applied by means of a pneumatic pot sprayer at a pressure of 2 bar in 200 L ha^{-1} of water.

3.2.4 Data collection

One set of control plants was harvested at the time of spraying and the following variables were recorded:

a. Number of leaves per plant

The number of leaves per plant were counted and recorded. The mean number of leaves of the four plants was then used as the number of leaves per experimental unit.

b. Leaf area (cm^2)

Leaf area per plant was measured using a leaf area meter (Li Cor) and the mean leaf area of the plants in a pot was then calculated per experimental unit.

c. Plant height (cm)

A calibrated ruler was used to measure plant height of each plant. The height considered was of the stems and leaves from the soil surface to the tip of the longest leaf (above root). The mean plant height per pot was then calculated.

d. Wet biomass (g)

From each pot, plants were harvested at the soil surface by means of secateurs and put into paper bags and the wet biomass was then measured using an electronic balance and recorded. Wet biomass was expressed as biomass per pot.

e. Dry biomass (g)

After determining the wet biomass, the paper bags with plants were put into an oven and dried at 80ºC for 48 hours. The dry plants were then weighed on an electronic balance and the dry mass per pot was then calculated.

A period of six weeks was allowed before evaluations were recorded to ensure that no regrowth of the plants took place as was previously observed with glyphosate resistant ryegrass. The following variables were recorded:

a. Percentage mortality (control)

Percentage mortality was recorded six weeks after spraying. The calculation was done using the following formulae;

$$\text{Percentage mortality} = \frac{number\ of\ dead\ plants\ per\ pot}{4(plants\ per\ pot)} \times 100\%$$

b. Dry matter of surviving ryegrass expressed as percentage of the control plants

The dry mass of the surviving plants in the sprayed pots that was still green were recorded in the same way as described above. The plants were green and live at the time of evaluation. Dry matter of surviving ryegrass was calculated as a percentage of unsprayed control plants dry matter.

The calculation was done using the following formulae;

$$\text{Dry matter of surviving ryegrass expressed as control percentage} = \frac{live\ dry\ mass\ per\ pot\ at\ evaluation\ (g\ )}{average\ live\ dry\ matter\ per\ pot\ (g)\ of\ control\ plants} \times 100\%$$

3.2.5 Data analysis

Data were subjected to an analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Bonferroni test for control variables recorded at spraying time and Fischer's $LSD_{0.05}$ for the data variables recorded at evaluation (six weeks after spraying). Bonferroni confidence intervals for differences of the means are wider than that of Fisher's LSD, therefore, Bonferroni test was used for one-way ANOVA of control variables whilst Fischer's $LSD_{0.05}$ was used for the factorial ANOVA.

3.3 RESULTS

There was significant (P-value ≤ 0.0001) interaction of temperature, age and dosage rate on the percentage mortality of ryegrass (See ANOVA table in Appendix 1). Generally, for both young plants and mature plants percentage mortality increased as the dosage rate of glufosinate ammonium increased at all four temperatures. However, for young plants growing at the two extreme temperatures (10/15 and 25/30 ºC), the 1.5 L ha^{-1} dosage rate resulted in significantly lower control percentages compared to 15/20 and 20/25 °C temperatures (**Figure 3.1**). At 3 L ha^{-1} however, control at 10/15 ºC was similar to the control provided 15/20 and 20/25 °C temperatures and this stayed the same up to the 7.5 L ha^{-1} dosage rate. At 25/30 ºC the 3 L ha-1 dosage rate also resulted in significantly lower control percentages than at the other temperatures. From 4.5 L ha^{-1} no significant difference was recorded between the percentage control attained at the different temperatures; at 6 L ha^{-1}, 100% control was achieved at all temperatures. At dosage rates of 3 L ha^{-1} and higher, glufosinate ammonium applied to plants growing at the cooler temperature of 10/15 ºC showed trends of better control although the differences were not statistically significant.

In mature ryegrass, glufosinate ammonium efficacy is better at 10/15 ºC where it was the only temperature achieving 100% ryegrass control with a dosage rate of 4.5 L ha^{-1} (**Figure 3.1**). Both the warmer temperatures, particularly 25/30 ºC, resulted in lower efficacy of glufosinate ammonium at the lower dosage rates. Glufosinate ammonium was more effective in controlling young ryegrass as compared to mature ryegrass.

Interaction of temperature, ryegrass growth stage and dosage rate on percentage dry matter of surviving ryegrass was significant with a P-value ≤ 0.0001 (See ANOVA table in appendix 2). Dry matter production of young ryegrass showed that even if mortality rates of

plants at 10/15, 15/20 and 20/25 °C night and day temperatures were greater than 80%, the dry matter of surviving ryegrass expressed as a percentage of the unsprayed control plants was not significantly different from 0% (**Figure 3.2**). This proved that even though there was survival, ryegrass plants were very small. A 100% control level of ryegrass in terms of dry matter of live ryegrass at 25/30 °C was only achieved with a dosage rate of 6 L ha^{-1}. Dry matter production of live mature ryegrass reached 0% for 10/15 °C using a dosage rate of 3 L ha^{-1} while the rest of the temperatures required a dosage rate of 6 L ha^{-1} to achieve the same results. Generally, control of mature ryegrass increased as temperature decreased.

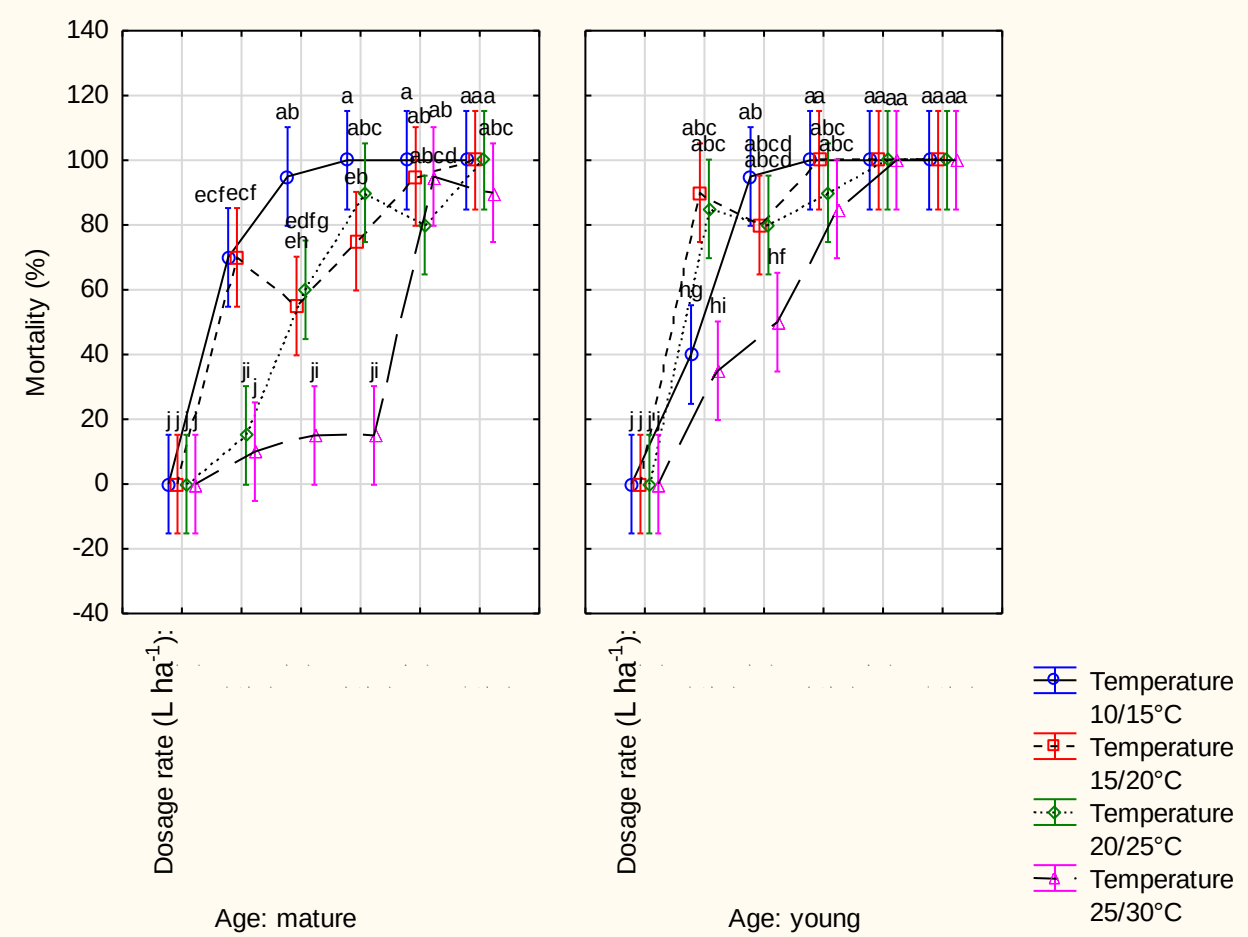

Figure 3.1: Mortality rates of young and mature ryegrass after application of various glufosinate ammonium dosage rates at four different temperatures. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

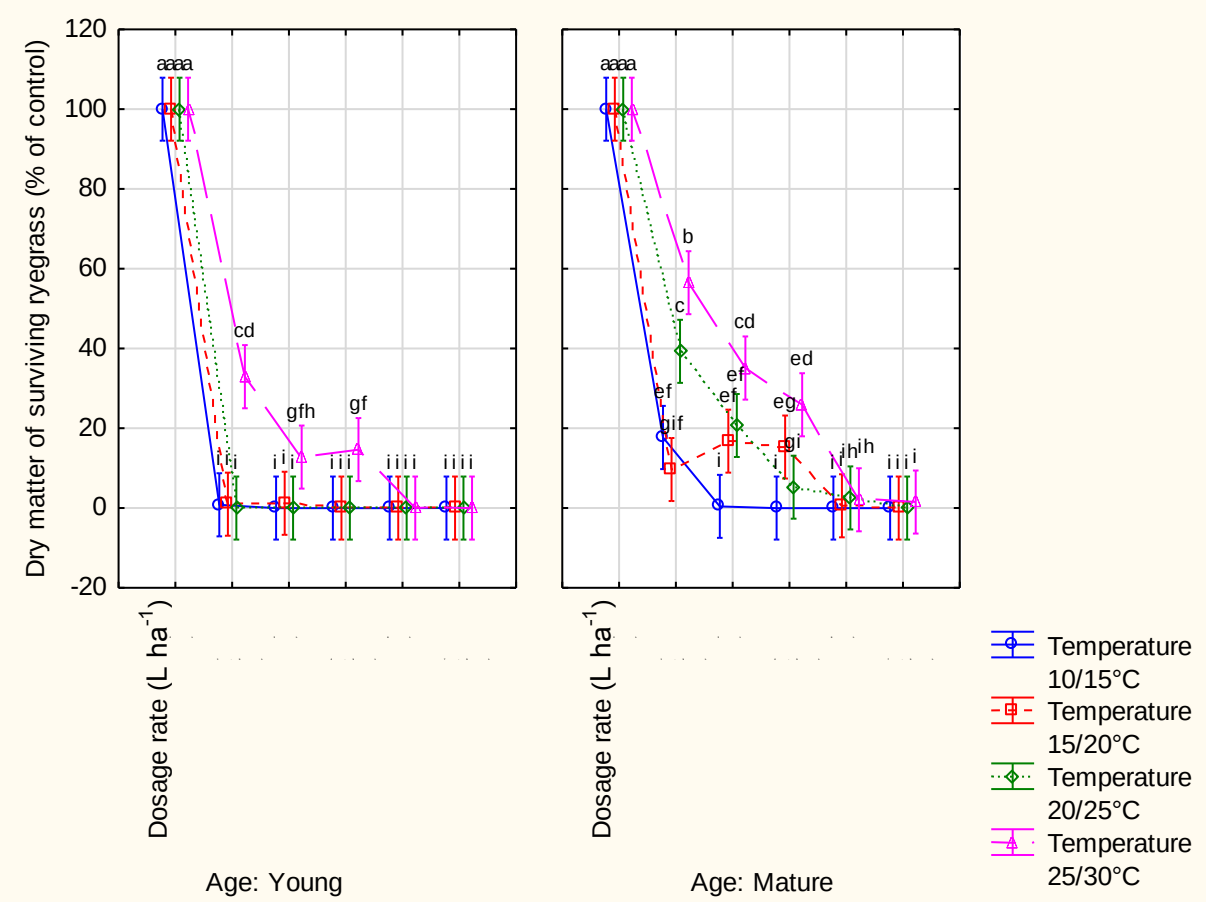

Figure 3.2: Dry matter of surviving ryegrass expressed as a percentage of the unsprayed control after application of various glufosinate ammonium dosage rates at two different plant growth stages. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

The optimum growing temperature for the ryegrass plants was at the 20/25 ºC temperature where most of the parameters were significantly higher compared to the other temperatures (**Table 3.2).**

Table 3.2: Vegetative growth parameters of control ryegrass plants growing at different temperatures at the time of spraying

	MATURE				YOUNG			
Temperature	**10/15°C**	**15/20°C**	**20/25°C**	**25/30°C**	**10/15°C**	**15/20°C**	**20/25°C**	**25/30°C**
Leaf number *	10.8^{b}	11.8^{c}	14.8^{a}	11.4^{b}	3.2^{d}	4.4^{d}	3.8^{d}	3.6^{d}
Leaf area (cm^2)	20.3^{c}	19.6^{c}	70^{a}	40.5^{b}	3.7^{d}	5.6^{d}	7.7^{d}	6.2^{d}
Plant height (cm)	19.1^{c}	23.2^{c}	34.1^{a}	28.18^{b}	10.2^{d}	15.4^{d}	13.9^{d}	12.7^{d}
Fresh weight (g)	2.89^{c}	2.43^{c}	10.21^{a}	5.86^{b}	0.32^{d}	0.55^{d}	0.63^{d}	0.44^{d}
Dry weight (g)	0.34^{c}	0.42^{c}	0.81^{b}	1.07^{a}	0.08^{d}	0.09^{d}	0.11^{d}	0.06^{d}

*Values followed by the same letter in a row do not differ significantly at $P \leq 0.05$

3.4 DISCUSSION

In general, control of ryegrass increased as dosage rate of glufosinate ammonium increased. This was observed for both mature and young ryegrass. According to Smeda and Putnam (2010) an increase of control as dosage rates increases is the expected results for herbicides. Control of 80% of young ryegrass at 15/20 and 20/25 °C required 4.5 L ha^{-1}, whereas a dosage rate of 3 L ha^{-1} controlled young ryegrass at 10/15 °C. For mature ryegrass, 6 Lha^{-1} was the effective dosage rate for all temperatures except at 10/15 °C where control of 95% was observed with a dosage rate of 3 L ha^{-1}. Effective dosage rates of 4.5 L ha^{-1} for young ryegrass and 6 L ha^{-1} for mature ryegrass correspond to the recommended dosage rate of 3-7 L ha^{-1} of Basta® (Bayer 1961). However, efficacy of glufosinate ammonium at higher dosage rate of 6 and 7.5 L ha^{-1} was not influenced by different temperatures.

In previous studies, it was proven that glufosinate ammonium controls weeds more effectively with increasing temperature (Pline et al. 2000; Kumaratilake and Preston 2005; Everman 2008; Everman et al. 2009). Contrary to these studies, the current study observed that generally, a lower temperature of 10/15 °C resulted in higher control of ryegrass for both mature and young plants as compared to 15/20, 20/25 and 25/30 °C night/day temperatures. For young plants, the variables recorded at the time of spraying had no significant difference between all temperatures. This showed that the different mortality rates at different temperatures observed when the plants were young cannot merely be attributed to plant factors, but to temperature. Poor control of ryegrass at 25/30 °C could be explained by

accumulation of relatively high dry matter, while the fresh weight was low. It is likely that the grass was moisture stressed.

Mature control plants growing in the different temperature regimes at the time of spraying had variables which were significantly different from each other among temperatures (**Table 3.2**). The significantly poorer control of ryegrass at 20/25 ºC and 25/30 ºC compared to the 10/15 ºC and 15/20 ºC temperatures at a dosage rate of 1.5 Lha^{-1} may be attributed to the bigger size of the plants at time of spraying. Growth stage however does not explain the better control of ryegrass plants growing at 20/25 ºC compared to those growing at 25/30 ºC for dosage rates of 3 and 4.5 L ha^{-1}. Ryegrass control at 10/15 and 15/20 °C temperature regimes were also not significantly different except for leaf number. Therefore, the significantly better control of ryegrass by glufosinate ammonium at 10/15°C compared to that of 15/20 °C for dosage rates of 3 and 4.5 L ha^{-1} cannot be attributed to plant size. The western Cape growing season is characterized by cool temperatures with day temperatures hovering around 16 °C, hence, glufosinate ammonium should effectively control ryegrass under these conditions.

Growth stage significantly influenced the control of ryegrass with glufosinate ammonium. Control of ryegrass was much more difficult in the case of mature ryegrass as explained in section 3.3. This observation can be ascribed to reduced absorption and translocation of glufosinate ammonium. Mature ryegrass control treatments' plants had higher leaf area as compared to the young ryegrass at the time of spraying (**Table 3.2**). Higher leaf area in plants depicts that the plants were bigger than the younger plants hence metabolic activity was probably slower which might have resulted in poor control of ryegrass (Weraduwage et al. 2015).

Efficacy of glufosinate ammonium in control of both young and mature ryegrass at lower temperatures was significantly greater than at higher temperatures. Studies have shown that as temperatures increase, plants modify their structure to reduce transpiration rate (Varanasi et al. 2015). The plants develop thick waxy cuticles which in turn reduce absorption and eventually translocation of herbicides (Jamal 2011). Under stress conditions, metabolism of plants decrease. Glufosinate ammonium mode of action entirely depends on metabolism of plants to produce optimum results. Development of a waxy cuticle on ryegrass and plant stress could have been reasons why control was significantly poorer at higher temperatures than at lower temperatures.

3.5 CONCLUSION

Glufosinate ammonium controlled ryegrass better at lower temperatures than at higher temperature. The most effective overall dosage rate for controlling young ryegrass was 4.5 L ha^{-1}, while 6 L ha^{-1} dosage rate was effective for mature ryegrass. However, 3 and 4.5 L ha^{-1} glufosinate ammonium dosage rates controlled both young and mature ryegrass at 10/15 °C. At higher dosage rates of 6 and 7.5 L ha^{-1}, temperature had no significant effect on glufosinate ammonium efficacy There was a significant effect of ryegrass growth stage on efficacy of glufosinate ammonium. When sprayed at the same rate under the same temperature, control of young ryegrass was 30% better than that of mature ryegrass.

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CHAPTER 4

EFFICACY OF GLUFOSINATE AMMONIUM AND AMS (AMMONUM SULPHATE) ON RYEGRASS AS INFLUENCED BY DIFFERENT TEMPERATURES

ABSTRACT

A glasshouse experiment was conducted at Welgevallen experimental farm to evaluate the efficacy of glufosinate ammonium with added adjuvant ammonium sulphate as influenced by different temperatures. The experiment was carried out on commercial ryegrass (*Lolium multiflorum* cv Energa) and the grass was sprayed 6 weeks after planting. Applied glufosinate ammonium dosage rates were 1, 2 and 3 L ha^{-1} with added ammonium sulphate at rates 1, 2 and 3%. Glasshouses were set at 10/15, 15/20, 20/25 and 25/30 °C night/day temperatures. The findings of the study indicated that a dosage rate of 3 L ha^{-1} glufosinate ammonium with addition of 2 and 3% ammonium sulphate controlled ryegrass effectively at the lower temperatures but not at 25/30 °C. Efficacy of glufosinate ammonium increased across all concentrations of AMS at lower temperatures compared to higher temperatures. An increase in AMS concentration resulted in increase of ryegrass control at lower temperatures but addition of AMS did not have a significant effect on the control of ryegrass at high temperatures.

Keywords: adjuvant, ammonium sulphate, dosage rate, glufosinate ammonium, ryegrass, temperature.

4.1 INTRODUCTION

The use of additives such as crop oils and a large range of wetting agents have not shown to assist in the performance of glufosinate ammonium. However, there are cases in which glufosinate ammonium has shown inconsistences in controlling weeds, hence, phytotoxity can be improved by adding adjuvants (Pratt et al. 2003). Despite the fact that it works best without additives, it has been noted that ammonium sulphate (AMS) improves uptake and efficacy of glufosinate ammonium in some situations (Bayer n.d).

Pratt et al. (2003) noted that the same principle in which AMS increases efficacy of glyphosate can be articulated for glufosinate ammonium, since the two herbicides have similar structures. Glyphosate readily reacts with calcium cations and other cations to form a

salt that is less soluble and poorly absorbed by plants. The sulphate anion from AMS reacts with the calcium cations to form calcium sulphate, thus allowing the ammonium ion to form readily absorbed NH_4-glyphosate molecule, hence, restricting the calcium cations from binding with the herbicide molecule.

Enhanced efficacy of herbicides is attributed to translocation of herbicides and differences in metabolism of plants that are grown under different temperatures (Kumaratilake and Preston 2005; Penner 2015). Contact herbicides like glufosinate ammonium translocate very little or not at all from the point of entry, however, under favorable conditions, limited movement occurs (Rao 2000; Kumaratilake and Preston 2005; Everman et al. 2009). The study done by Pratt et al. (2003) proved that AMS induced favorable conditions for glufosinate ammonium to be absorbed and be translocated effectively into the plant.

Results in Chapter 3 showed that high temperatures reduce efficacy of glufosinate ammonium. With the aim to observing if AMS enhances efficacy of glufosinate ammonium, the specific objective of this study was to the determine the effect of three concentrations of the adjuvant AMS and temperature on glufosinate ammonium efficacy on ryegrass. The overall null hypothesis was that temperature and AMS concentration do not influence glufosinate ammonium efficacy on ryegrass.

4.2 MATERIALS AND METHOD

4.2.1 Experimental site

The experiment was conducted in a glasshouse at the Stellenbosch University Welgevallen Experimental Farm. The site is located at 33° 56´33" S and 18° 51´56" E and at an altitude of 136 m above sea level.

4.2.2 Treatments and experimental design

The study was conducted in glasshouses set at 10/15, 15/20, 20/25 and 25/30 °C night/day temperatures. The design was a 3× 4 × 3 factorial arranged in a randomized complete block design with 6 replications. The experimental factors were different AMS concentrations (1, 2, and 3 %), temperature at four levels (10/15, 15/20, 20/25 and 25/30 °C night/day) and glufosinate ammonium dosage rates at three levels (1, 2 and 3 L ha^{-1}).

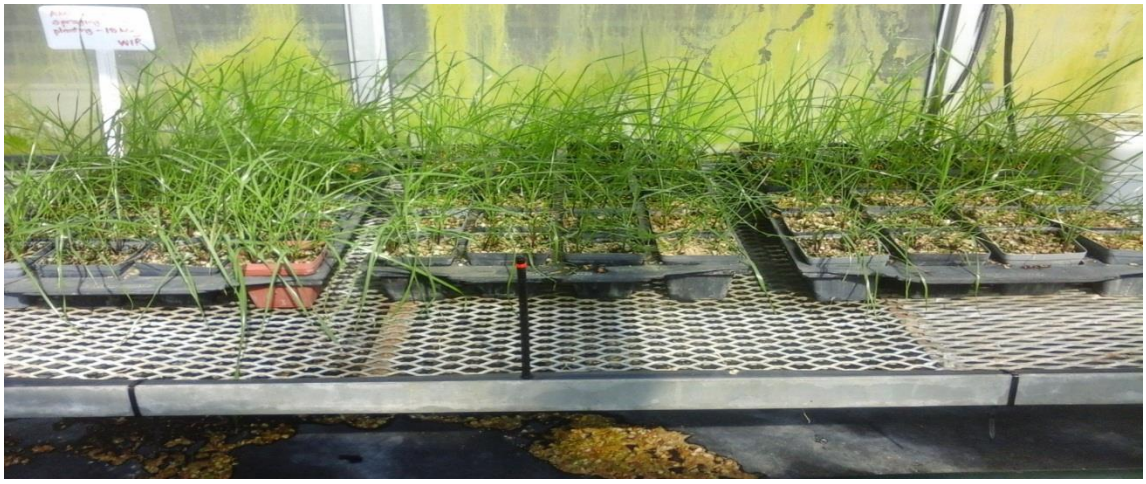

Figure4.1: Ryegrass in pots prior to spraying

4.2.3 Trial establishment and management

Planting

Ryegrass (*Lolium multiflorum* cv Energa) seeds were first germinated in petri dishes in an incubator at 20 ºC under light conditions to ensure higher percentage germination and the resulting seedlings were transplanted into 8 x 8 cm square plastic pots after 2 weeks.

Fertilization

A nutrient solution was used to fertilize ryegrass plants during the study. The composition is shown in **Table 4.1**.

Table 4.1: Composition of the nutrient solution used to fertilize the plants growing in pots

EC = 2.0			
Element (Macro)	Concentration mg L^{-1}	Fertilizer	Concentration g $1000L^{-1}$
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$N0_3^-$	661.33	$MgS0_4.7H_20$	492
H_2P0_4	116.4	KH_2P0_4	136
$S0_4$	390.4		
(Micro)	mg L^{-1}		
Fe: Libfer (Fe EDTA)	0.85		6.54
Mn: Manganese sulphate	0.55		2.23
Zn: Zinc sulphate	0.30		1.33
B: Solubor	0.30		1.46
Cu: Copper Sulphate	0.05		0.20
Mo: Sodium Molibdate	0.02		0.13

Weed, pest and disease control

Additional weeds were removed by hand. No pests and diseases were experienced in the glasshouse.

Irrigation

An automated irrigation system was used to water the plants. The plants were irrigated at 8:00 am, 12:00 pm, 2:00 pm and 4:00 pm. The quantity of water per irrigation was adjusted depending on the plant growth stage to compensate for increased usage by bigger plants.

Herbicide application

Ryegrass was sprayed with glufosinate ammonium at four weeks after planting. The herbicide was applied by means of a pneumatic pot sprayer at a pressure of 2 bar at 200 L ha^{-1} of water.

4.2.4 Data collection

One set of control plants was harvested at the time of spraying and the following variables were recorded;

a. Number of leaves per plant

The number of leaves per plant were counted and recorded. The mean number of leaves of the four plants was then used as the number of leaves per experimental unit.

b. Leaf area (cm^2)

Leaf area per plant was measured using a leaf area meter (Li Cor) and the mean leaf area of the plants in a pot was then calculated.

c. Plant height (cm)

A calibrated ruler was used to measure plant height. The height considered was of the stems and leaves (above root) from the soil surface to the tip of the longest leaf. The mean plant height per pot were then calculated.

d. Wet biomass of alive and dead matter (g)

From each pot, plants were harvested at the soil surface by means of secateurs and put into paper bags to determine wet biomass using an electronic balance. Wet biomass was expressed as biomass per pot.

e. Dry biomass of alive and dead matter (g)

After determining the wet biomass, the paper bags with plants were dried at 80ºC for 48 hours. Samples were weighed on an electronic balance and the dry mass per pot was calculated.

A period of six weeks allowed before evaluation was selected to ensure that no regrowth of the plants take place as was previously observed with glyphosate resistant ryegrass.

a. Percentage mortality (%)

Percentage mortality was recorded six weeks after spraying. The calculation was done using the following formulae;

$$\text{Percentage mortality} = \frac{number\ of\ dead\ plants\ per\ pot}{4(plants\ per\ pot)} \times 100\%$$

b. Dry matter of surviving ryegrass (g)

The dry mass of the green, surviving plants in the sprayed pots were recorded after drying the plants in the oven at 80ºC for 48 hours.

4.2.5 Data analysis

Data were subjected to an analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Bonferroni test for control variables recorded at spraying time and Fischer's $LSD_{0.05}$ for the data variables recorded at evaluation (six weeks after spraying). Bonferroni confidence intervals for differences of the means are wider than that of Fisher's LSD, therefore, Bonferroni test was used for one-way ANOVA of control variables whilst Fischer's $LSD_{0.05}$ was used for the factorial ANOVA.

4.3 RESULTS

The ANOVA table of percentage mortality showed a significant interaction of AMS concentration, temperature and glufosinate ammonium dosage rates (P-value = 0.01) (see table in Appendix 3). An increase in AMS concentration increased the efficacy of glufosinate ammonium and this was more noticeable for temperature 10/15 °C at a dosage rate of 1 L ha^{-1} (**Figure 4.2**). The effect of AMS at the same dosage rate at the other temperatures was erratic and showed no clear trends.

There was a general trend of increase in efficacy of glufosinate ammonium as the temperature increased from 10/15 °C to 15/20 °C but a gradual decrease of efficacy was also

observed with higher temperatures of 20/25 and 25/30 °C (**Figure 4.2**). The highest percentage mortality observed with glufosinate ammonium dosage rate of 1 L ha^{-1} and 1% AMS concentration was approximately 80% under 15/20 °C, while dosage rates of 2 L and 3 L ha^{-1} resulted in control not significantly different from 100% at 10/15, 15/20 and 20/25 °C temperatures. Poor efficacy of glufosinate ammonium is shown at a temperature of 25/30 °C using a dosage rate of 1 and 2 L ha^{-1}, however, a dosage rate of 3 L ha^{-1} resulted in percentage mortality that was not significantly different from 100%.

The 2% AMS concentration showed a decrease in efficacy of glufosinate ammonium with increasing temperature at 1 L ha^{-1}(**Figure 4.2**). The data was inconsistent with no fixed pattern. A dosage rate of 1 L ha^{-1} showed variable control of ryegrass with a low percentage of approximately 65% at 15/20 °C temperature. Even though mortality caused by a dosage rate of 2 Lha^{-1} varied from 80 to 100%, there were no statistical differences between the temperatures. A steady percentage mortality of 100% is observed at a dosage rate of 3 L ha^{-1}.

A similar trend to that of 1% AMS concentration was observed for the 3% AMS concentration in which there was a general decrease in efficacy of glufosinate ammonium at high temperatures (**Figure 4.2**). A slight difference was observed in the curve of the dosage rate of 1 L ha^{-1} where a steep decrease of efficacy was observed 15/20 °C temperature. Herbicide dosage rates of 2 and 3 L ha^{-1} had a consistent 100% control of ryegrass at 10/15 and 15/20 °C night/day temperatures. However, a slight decrease in the efficacy was observed at a temperature of 25/30 °C for both herbicide dosage rates.

For all the variables measured at time of spraying, the P-values were less than 0.01 indicating significant differences in means (**Table 4.2**). The greatest difference in means of all variables was observed at 15/20 °C compared to the rest of the temperatures. Ryegrass grows better at 15/20 °C (**Table 4.2**). However, leaf number and fresh weight of ryegrass at 10/15 and 20/25 °C showed no significant difference at 15/20 °C temperature.

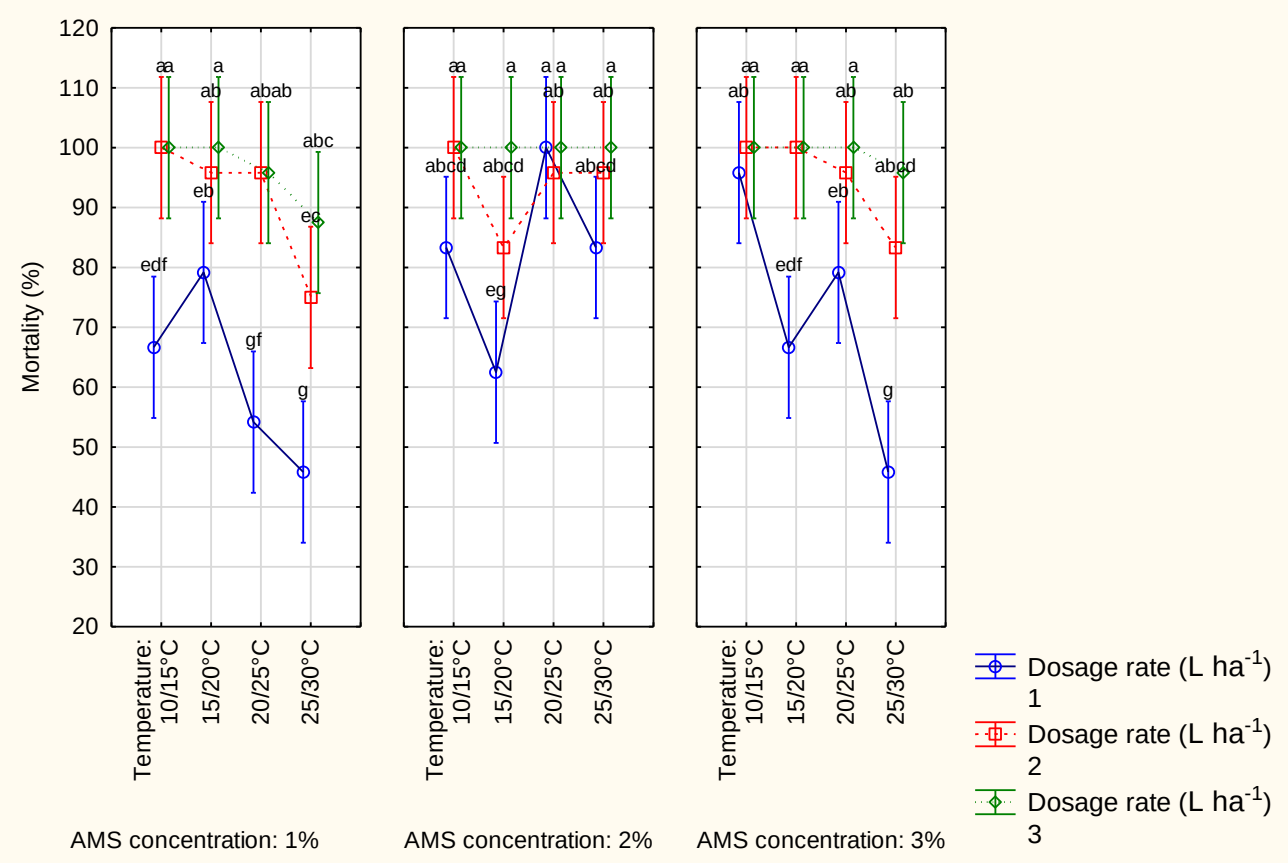

Figure 4.2: Mortality rates of ryegrass plants after application of different glufosinate ammonium dosages and AMS concentrations. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

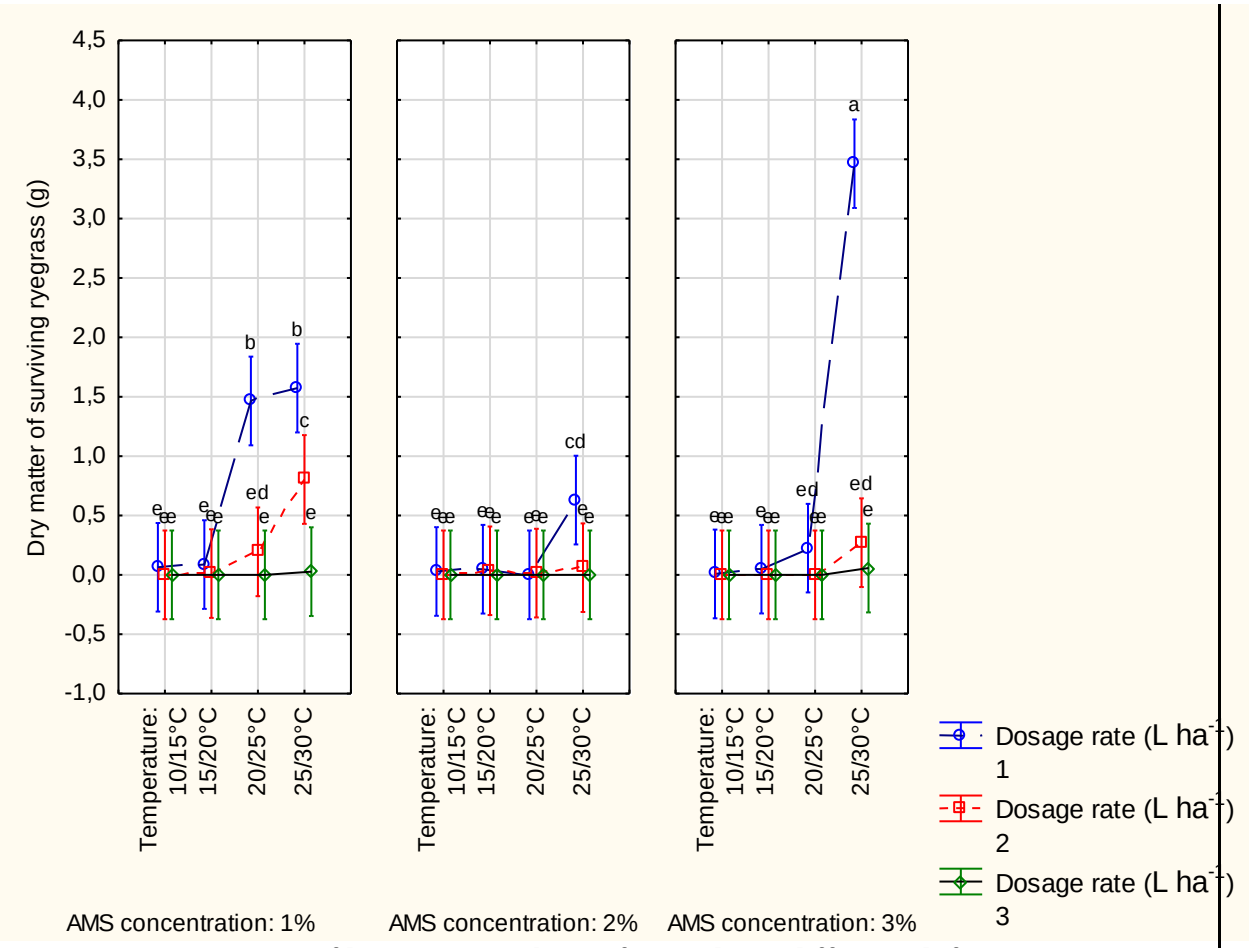

Figure 4.3: Dry matter of live ryegrass plants after applying different glufosinate ammonium dosages and AMS concentrations. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

Table 4.2: Vegetative growth parameters of control ryegrass plants growing at different temperatures at the time of spraying

Temperature	**10/15 °C**	**15/20 °C**	**20/25 °C**	**25/30 °C**
Leaf number*	7.8 [a]	7.3 [a]	6 [ab]	5 [b]
Plant height	17.8 [b]	24.4 [a]	29.3 [a]	26.2 [a]
Leaf area	15.8 [b]	22.6 [a]	16 [b]	11.1 [b]
Fresh weight	2 [ab]	2.9 [a]	1.9 [ab]	1.2 [b]
Dry weight	0.27 [b]	0.46 [a]	0.16 [b]	0.14 [b]

*Values followed by the same letter in a row do not differ significantly at $P \leq 0.05$

Interaction of temperature, AMS concentration and dosage rates gave a significant P value ≤ 0.0001 (See ANOVA table in appendix 4). The 2% AMS concentration graph for dry matter of surviving ryegrass showed mortality of over 95% for all dosage rates and temperatures except for the 1 L ha^{-1} dosage rate at 25/30 °C (**Figure 4.3**). Survival was more noticeable at 20/25 and 25/30 °C as compared to lower temperatures for both 1 and 2% AMS graphs.

4.4 DISCUSSION

The study proved that a dosage rate of 3 L ha^{-1} with 1, 2 and 3% AMS concentration gave control of ryegrass that ranged from 90-100%. However, when using 1% AMS concentration at higher temperatures a lower mortality resulted when compared to the lower temperatures. It can be concluded that a glufosinate dosage rate of 3 L ha^{-1} is effective with AMS concentration of 2 and 3%. A 1% AMS concentration could be more feasible for a cool environment that is equal or less than 15 °C during the night and 20 °C during the day. A temperature of 25/30 °C might require glufosinate ammonium dosage rates higher than 3 L ha^{-1} to produce satisfactory control of ryegrass.

Regardless of some inconsistent results in control of ryegrass at different temperatures, the general resultant trend was a decrease in control of ryegrass as temperature increased. This was true for treatments with AMS 1, 2 and 3%. This observation can possibly be ascribed to the different accumulation rates of calcium concentration in ryegrass with different temperatures. Pratt et al. (2003) noted that an increase in calcium concentrations decreases efficacy of the glufosinate ammonium since it binds with the glufosinate molecule, and thus reducing its absorption into plants. An increase in environmental temperature results in a transient increase of cytoplasmic calcium ion concentration (Gong et al. 1998; Knight 2000; Knight and Knight 2001). It could be a possibility that the continuous exposure of ryegrass to high temperatures such as 20/25 and 25/30 °C led to a permanent high concentration of calcium ions. The concentration could have been high enough to significantly reduce the efficacy of glufosinate ammonium as compared to lower temperatures.

At lower temperatures of 10/15 and 15/20 °C, an increase in AMS concentration increased the efficacy of glufosinate ammonium. However, this trend was not evident for higher temperatures. This could be explained in terms of reduced bonding of calcium ions to the glufosinate molecule by an increase in sulphate ions that react with the calcium ions. This

explanation proved to be not true for ryegrass growing at higher temperatures. A possibility could be that the cytoplasmic calcium concentration at high temperatures is significantly higher and an increase of AMS concentration could not yield a noticeable change. Maybe a higher concentration of AMS can result in higher efficacy of glufosinate ammonium at high temperatures.

4.5 CONCLUSION

Glufosinate ammonium controlled ryegrass better at lower temperatures than at higher temperatures. Effective control of ryegrass was recorded across all concentrations of AMS at lower temperatures compared to higher temperatures. The omission of a control AMS treatment, where no AMS was added, detracts immensely to the value of these results. However, the results do confirm some aspects that were investigated and reported in Chapter 3 and as such do have some value. The possible role of Ca concentration in leaf tissue on the herbicide's efficacy, as well as the role of AMS in mitigating the Ca effect, deserve investigation.

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CHAPTER 5

THE EFFICACY OF GLUFOSINATE AMMONIUM ON RYEGRASS AS INFLUENCED BY DIFFERENT PLANT GROWTH STAGES

ABSTRACT

Glasshouse and field experiments were conducted to determine the influence of different ryegrass (*Lolium multiflorum* cv Energa) growth stages on glufosinate ammonium efficacy. Glasshouse experiments were conducted at Welgevallen experimental farm and the field experiments were conducted at Welgevallen, Roodebloem and Langgewens experimental farms. Application of glufosinate ammonium was done at 2-week intervals from the planting date in the field to accommodate five levels of growth stages (2, 4, 6, 8 and 10 weeks) within 10 weeks. Two methods were used in the glasshouse to obtain different growth stages. One method was similar to the field and the second one involved planting ryegrass at 2-week intervals and then spraying once after 12 weeks. Applied dosage rates were 1.5, 3, 4.5, 6 and 7.5 L ha^{-1} for glasshouse experiments and 2.5, 5, 7.5 and 10 L ha^{-1} for field experiments. Results of this study proved that growth stage of ryegrass has no influence on efficacy of glufosinate ammonium. However, differences in control were observed for different glufosinate ammonium dosage rates. The study also observed higher control of ryegrass in the glasshouse as compared to control in the field.

Key words: dosage rate, field experiment, glasshouse experiment, glufosinate ammonium, growth stage, ryegrass.

5.1 INTRODUCTION

Weed growth stages influence plant size, plant surface area, cuticle composition and the source to sink relationship (Chism et al. 1992). All these factors account to the efficacy of herbicides including glufosinate ammonium after application on weeds (Chism et al. 1992; Steckel, et al. 1997b) An ARC annual report (2013/2014) noted that billions of rands have been spent towards control of ryegrass (*Lolium spp*.). Ryegrass persistence is mainly due to its ability to undergo natural mutation, develop dense stands and establish itself earlier than all species, gaining competitive advantages and causes significant yield losses in several crops (Todd 2008).

Rosales-Robels et al. (1999) noted that the critical period of weed competition for crops is the first 3 to 8 weeks of their development to prevent significant yield loss. Generally, herbicides are more effective on younger weeds than on mature weeds (Ahmadi et al. 1980). Mellendorf et al. (2013) studied the efficacy of glyphosate on horseweed (*Coryza canadensis*) and observed that the regression model predicted 40-58% control for weeds at a height above 15 cm and declines to 15-39% when the height increased to 45 cm. The same experiment found that plant height that ranged from 5 cm to 15 cm gave control of glyphosate greater than 94%.

Use of glufosinate ammonium as a post-emergent herbicide has great potential in controlling ryegrass in crops where it can be applied. However, efficacy of the herbicide is highly dependent on environmental and plant factors. The objectives of this research were (i) to determine the effective dosage rate of glufosinate ammonium in controlling ryegrass, (ii) to observe and determine the influence of different ryegrass growth stages on efficacy of glufosinate ammonium in the field and glasshouse and (iii) to compare two different methods of obtaining different growth stages in the glasshouse.

5.2 MATERIALS AND METHOD

5.2.1 Glasshouse experiment

The experiment was conducted in a glasshouse at Stellenbosch University Welgevallen experimental farm, 33° 56´33" S and 18° 51´56" E at an altitude of 136m above sea level. Commercial ryegrass (*Lolium multiflorum* cv Energa) was grown in pots. The study was conducted in a glasshouse at 18/23℃ night/day temperatures. The design was a 5 × 6 factorial arranged in a randomized complete block with 5 replications. The experimental factors were plant size at 5 levels (2, 4, 6, 8 and 10 weeks) and glufosinate ammonium dosage rates at five levels (0, 1.5, 3, 4.5, 6 and 7.5 L ha^{-1}). Two methods of obtaining different sized plants were used. For the first method, ryegrass was planted simultaneously in all the pots at the time the experiment was initiated and was sprayed at 2-week intervals from sowing date to the 10^{th} week. For the second method, ryegrass in the pots was sown at 2-week intervals from the beginning of the experiment until the 10^{th} week and all the pots were sprayed simultaneously after 12 weeks. After spraying, ryegrass was left inside the spraying room overnight to prevent the herbicide being washed off the leaves by irrigation treatments.

Seed was sown into 2 L plastic containers filled with potting sand to germinate. Seedlings were transplanted into 8 x 8 cm square plastic pots one week after the sowing date. A nutrient

solution was used to fertilize ryegrass plants during the study. The composition is shown **Table 5.1**. Additional weeds were removed by hand. No pests and diseases were experienced in the glasshouse. An automated irrigation system was used to water the plants. The plants were irrigated at 8:00 am, 12:00 pm, 2:00 pm and 4:00 pm. The quantity of water per irrigation was adjusted depending on the plant growth stage and weather conditions to compensate for water loss. The herbicide was applied by means of a pneumatic pot sprayer at a pressure of 2 bar in 200 L ha^{-1} of water. For the first method, 2-, 4-, 6-, 8- and 10-week old plants were sprayed on 24 February, 10 March, 23 March, 6 April and 20 April 2016 respectively. For the second method, spraying was done once on the 20th of April 2016. Evaluation was done four weeks after the spraying date.

Table 5.1: Composition of the nutrient solution used to fertilize the plants growing in pots

EC = 2.0			
Element (Macro)	Concentration mg L^{-1}	Fertilizer	Concentration g 1000L^{-1}
K^{+}	237.7	$KN0_3$	303
Ca^{++}	180	K_2S0_4	261
Mg^{++}	48.6	$Ca\ (N0_3)_2.\ 2H_20$	900
$N0_3^{-}$	661.33	$MgS0_4.7H_20$	492
H_2P0_4	116.4	KH_2P0_4	136
$S0_4$	390.4		
(Micro)	mg L^{-1}		
Fe	0.85	Libfer (Fe EDTA)	6.54
Mn	0.55	Manganese sulphate	2.23
Zn	0.30	Zinc sulphate	1.33
B	0.30	Solubor	1.46
Cu	0.05	Copper Sulphate	0.20
Mo	0.02	Sodium Molibdate	0.13

5.2.2 Field experiments

The trial layout was a 5 × 5 factorial design arranged in a randomized complete block with 4 replications. The experimental factors were plant size at 5 levels (2, 4, 6, 8 and 10 weeks) and glufosinate ammonium dosage rates at five levels (0, 2.5, 5, 7.5 and 10 L ha^{-1}). Commercial ryegrass seeds were sown in the field and grasses were sprayed every two weeks starting two weeks after the sowing date to 10 weeks after sowing. This experiment was repeated at three locations viz. Welgevallen, Roodebloem and Langgewens experimental farms. Langgewens experimental farm geographical location is 33°16'0" S and 18°42'0" E with an altitude of 144 m above sea level. Roodebloem experimental farm is located 34°9'0" S and 21°49'0" E geographically with an altitude of 155 m above sea level. Ten grass samples in control treatments were harvested at the time of spraying and the same variables as explained in section 5.3.2 were recorded for each plant. A CP3 spraying knapsack was used to spray

glufosinate ammonium in 200 L $ha^{-1}$of water. Spraying of 2-, 4-, 6-, 8- and 10-week old plants was done on 30 June, 14 July, 28 July, 11 and 18 August 2015 respectively. Data collection was done on the 11th of August, 25th August, 8th September, 22nd September and 6th October 2015, respectively at approximately 6 weeks after spraying.

5.2.3 Data collection

For both glasshouse and field experiments, control plants were harvested at the time of spraying and the following variables were recorded:

a. Number of leaves per plant

The number of leaves per plant were counted and recorded. In the glasshouse, the mean number of leaves of the four plants was then used as the number of leaves per pot.

b. Leaf area (cm^2)

Leaf area per plant was measured using a leaf area meter (Li Cor). The mean leaf area of the plants in a pot was then calculated for glasshouse experiments.

c. Plant height (cm)

A calibrated ruler was used to measure plant height. The height considered was of the stems and leaves (above root) from the soil surface to the tip of the longest leaf of each plant. The mean plant height per pot was calculated for the glasshouse experiments.

d. Wet biomass (g)

From each pot, plants were harvested at the soil surface by means of secateurs and put into paper bags and the wet biomass was then measured using an electronic balance and recorded. Wet biomass was expressed as biomass per pot for glasshouse experiments.

e. Dry biomass (g)

After determining the wet biomass, the paper bags with plants were put into an oven and dried at 80 ºC for 48 hours. The dry plants were then weighed on an electronic balance and the dry mass per pot was then calculated.

At four weeks after spraying the treated plants in the glasshouse were evaluated for mortality. The following variables were recorded;

a. Percentage mortality (%)

Percentage mortality was recorded four weeks after spraying. The calculation for glasshouse experiments was done using the following formulae;

Percentage mortality = $\frac{number\ of\ dead\ plants\ per\ pot}{4(plants\ per\ pot)} \times 100\%$

Percentage mortality in the field was determined by means of visual observations. Three individuals recorded independent visual observations, after which ratings were compared between treated and control plots to determine the percentage control of ryegrass. The average control was calculated from the three readings and used as the percentage mortality.

b. Dry matter of surviving ryegrass expressed as percentage of the control treatment

Dry matter of surviving ryegrass was calculated as percentage of the unsprayed control treatment on glasshouse plants. The plants were green and live at the time of evaluation. The calculation was done using the following formulae;

Dry matter of surviving ryegrass expressed as a control percentage =

$$\frac{dry\ mass\ of\ surviving\ sprayed\ plants\ (g)}{average\ \ dry\ mass\ of\ the\ unsprayed\ control\ plants\ (g)} \times 100\%$$

5.2.5 Data analysis

Data were subjected to an analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Bonferroni test for control variables recorded at spraying time and Fischer's $LSD_{0.05}$ for the data variables recorded at evaluation (six weeks after spraying). Bonferroni confidence intervals for differences of the means are wider than that of Fisher's LSD, therefore, Bonferroni test was used for one-way ANOVA of control variables whilst Fischer's $LSD_{0.05}$ was used for the factorial ANOVA.

5.3 RESULTS

5.3.1 Glasshouse experiments

A. Method 1- ryegrass planted once

Growth stage levels and glufosinate ammonium dosage rates had a significant interaction with P-value ≤ 0.0001 (see Appendix 5 for ANOVA table). In general, and across all the growth stages, control of ryegrass increased as dosage rate increased (**Figure 5.1**). Another observation was that control became less predictable as ryegrass became more mature (10 weeks). Ryegrass at growth stage of 2, 4, 6 and 8 weeks reached a mortality of 100% with 4.5 L ha^{-1} dosage rate. However, control of 6 -week old ryegrass reduced to 90% with 6 L ha^{-1} dosage rate. Ryegrass control at week 10 only achieved 100% control with a dosage rate of 7.5 L ha^{-1}. However, control of 10-week old ryegrass was not significantly different from the rest of the growth stages except when it showed an approximate mortality of 80% using a dosage rate of 4.5 L ha^{-1}. The 6-week old ryegrass was controlled significantly poorer than the rest of the treatments at 1.5 L ha^{-1}.

Table 5.2: Vegetative growth parameters of control ryegrass plants different growth stages in the glasshouse at the time of spraying (method 1)

Growth stage	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 10**
Leaf number*	7^{c}	14^{b}	21^{a}	19^{ab}	25^{a}
Leaf area (cm^2)	29.6^{c}	92.56^{b}	160.35^{a}	228.14^{a}	192.45^{a}
Plant height (cm)	22.1^{c}	33.1^{cb}	39.35^{ab}	48.5^{a}	46^{ab}
Fresh weight (g)	0.81^{d}	4.93^{c}	7.64^{cb}	9.8^{ab}	13.4^{a}
Dry weight (g)	0.076^{c}	0.4^{b}	1.133^{a}	1.46^{ab}	2.21^{a}

*Values in a row followed by the same letters indicate no significant differences between treatments at P ≤ 0.05

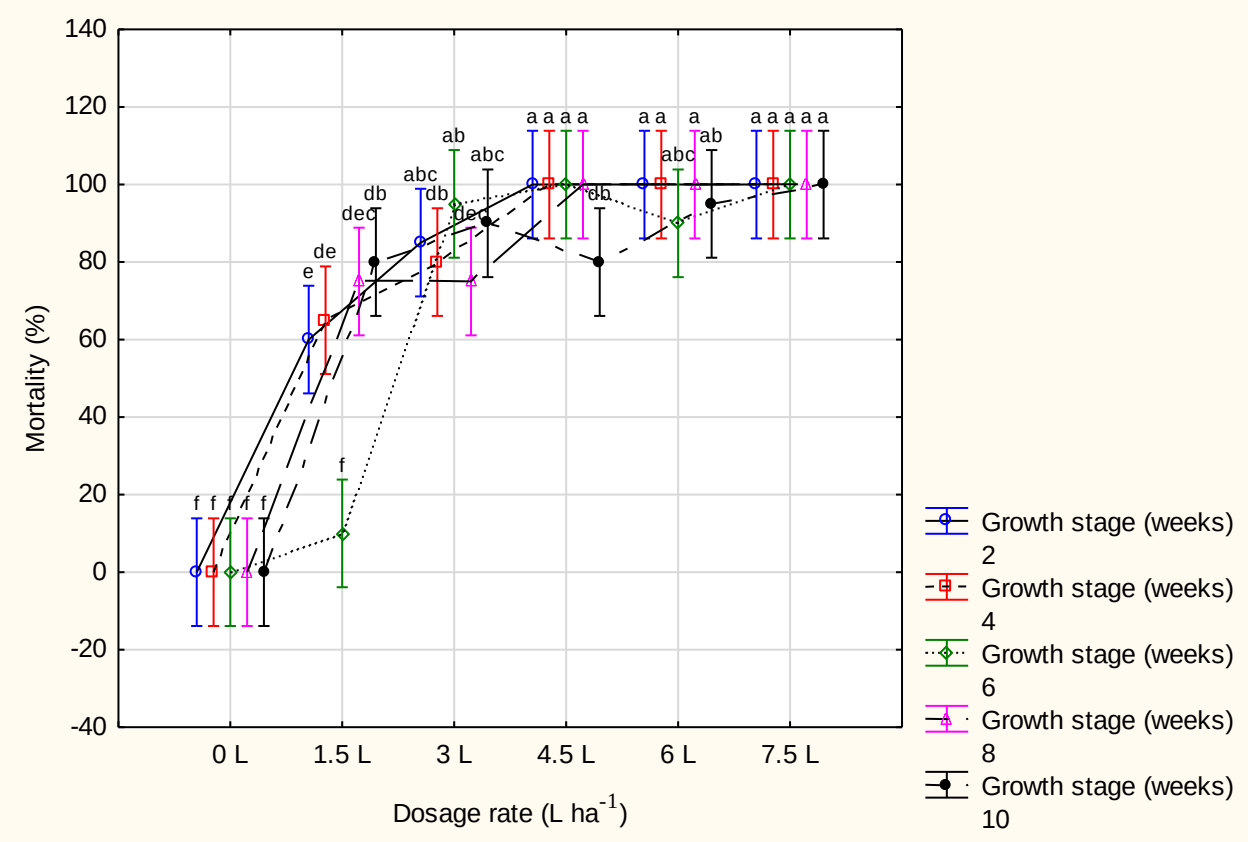

Figure 5.1: Glasshouse experiment 1: mortality rates of ryegrass after application of various glufosinate ammonium dosage rates at five different growth stages. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

Leaf number, leaf area, plant height and dry weight variables of 6-, 8- and 10-week old ryegrass plants showed that the largest plants were not significantly different from each other (**Table 5.2**). For the same parameters, 4-week old ryegrass plants were significantly different from the 6-, 8- and 10-week old and 2-week old ryegrass. Fresh weight of 4- and 6-week old ryegrass was not significantly different from each other as well as 8- and 10-week old. But, both were different from each other and 2-week old ryegrass.

There was a significant interaction between growth stage and dosage rate for percentage dry matter of surviving ryegrass with a P-value ≤ 0.0001 (See ANOVA table in appendix 6) The percentage dry matter of surviving ryegrass graph (**Figure 5.2**) showed that 100% mortality of 6-week old ryegrass was reached with dosage rate of 3 L ha^{-1}. A 100% mortality rate of 2- and 4-week old ryegrass was only reached with a dosage rate of 4.5 L ha^{-1}. 8- and 10-week old ryegrass mortality reached 100% with a dosage rate of 7.5 L ha^{-1} although

control of 8-week old ryegrass was significantly poorer with the percentage dry matter of 26%.

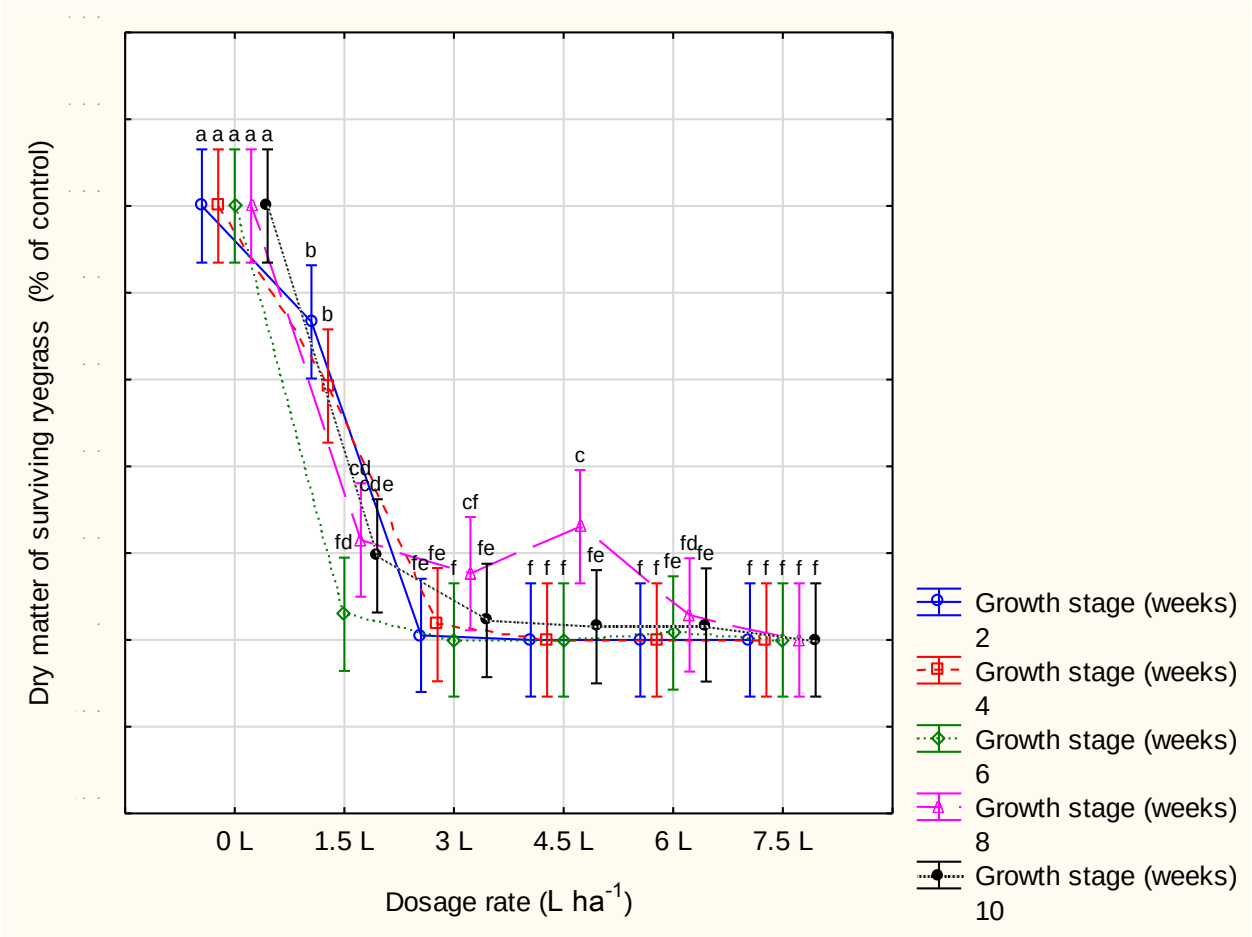

Figure 5.2: Dry matter of surviving plants as a percentage of the unsprayed control plants after application of various glufosinate ammonium dosage rates at five different growth stages for method 1. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

B. Method 2- ryegrass sprayed once

In method 2 there was an interaction between dosage rate and ryegrass growth stage (P-value ≤ 0.0001) (see Appendix 7 for ANOVA table). A 100% mortality rate of ryegrass for 2-, 4-, 6- and 8-week old ryegrass was only achieved using a dosage rate of 6 L ha^{-1} **(Figure 5.3)**. Similarly, to the first method, control of 10-week old plants, control only reached 100% with 7.5 L ha^{-1} dosage rate. Control of 10-week old ryegrass followed the same trend as in the first method. Control of 2- and 4-week old ryegrass with 1.5 L ha^{-1} was exceptionally poor showing no significant difference from ryegrass in control treatments.

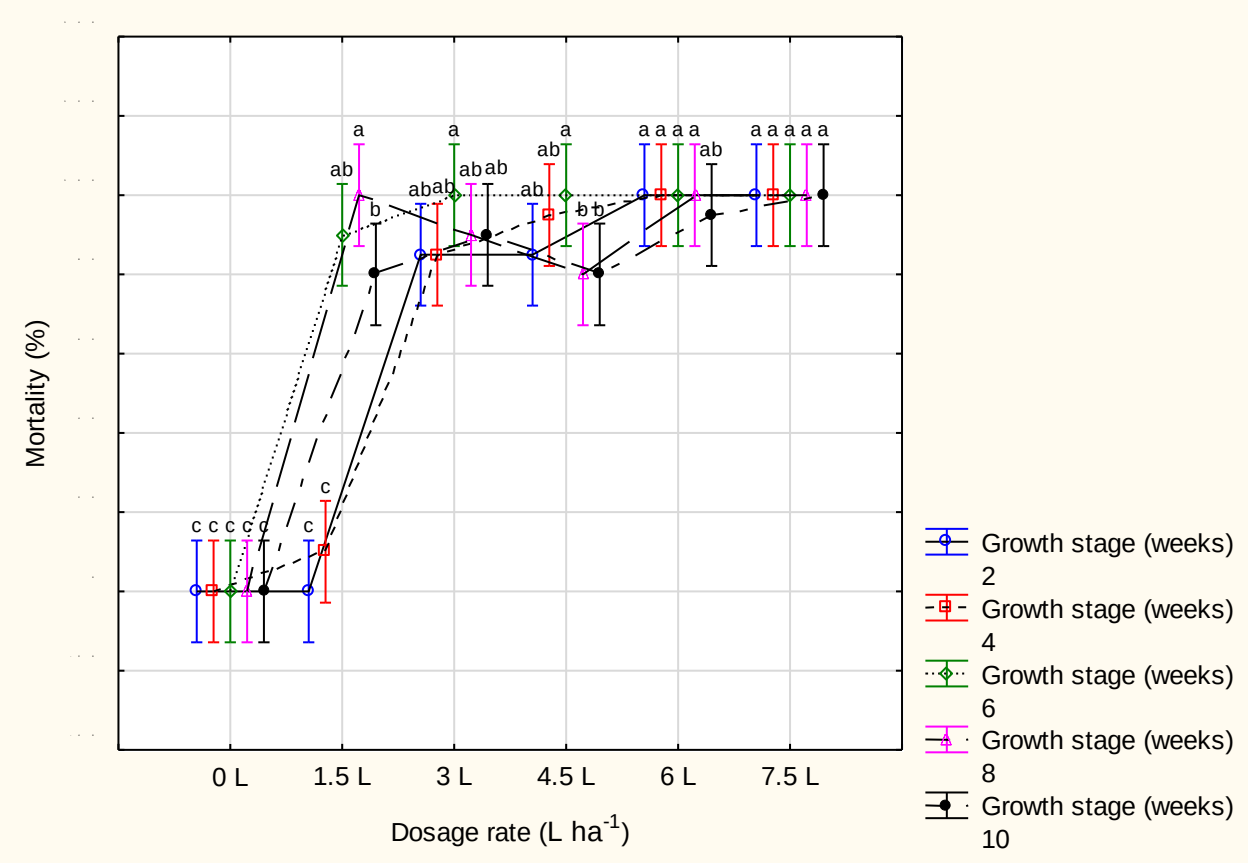

Figure 5.3: Glasshouse experiment method 2: mortality rates of ryegrass after application of various glufosinate ammonium dosage rates at five different growth stages. Means indicated by different letters differed significantly at P ≤ 0.05 according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

Leaf area and plant height of 6-, 8- and 10-week old ryegrass did not differ significantly (**Table 5.3**). Leaf number and leaf area of 2- and 4-week plants showed significant differences while the plant height, fresh weight and dry weight did not differ significantly. Accumulated dry matter in 8- and 10-week old plants was high and not significantly different from each other. The higher leaf area values of 6-, 8- and 10-week old ryegrass indicated that they were bigger plants compared to 2- and 4-week old plants.

There was a significant interaction of growth stage and dosage rate on percentage dry matter of surviving ryegrass with a P-value ≤ 0.0001 (See ANOVA table in appendix 8). Control of 6-week old ryegrass reached 100% with dosage rate of 1.5 L ha^{-1} only (**Figure 5.4**). 100% control of growth stages 2 and 4 achieved a 100% control with 4.5 L ha^{-1} while that of 10-week old ryegrass was achieved with 7.5 L ha^{-1} dosage rate.

Table 5.3: Vegetative growth parameters of control ryegrass plants at different growth stages in the glasshouse at the time of spraying (method 2)

Growth stage	Week 2	Week 4	Week 6	Week 8	Week 10
Leaf number*	6^{c}	9^{b}	13^{b}	18^{ab}	25^{a}
Leaf area (cm^2)	15.2^{c}	24.3^{c}	114.95^{ab}	161.7^{a}	192.45^{a}
Plant height (cm)	25^{c}	23.4^{c}	42.4^{ab}	50^{a}	46^{ab}
Fresh weight (g)	1.24^{c}	1.25^{c}	4.6^{cb}	9.02^{ab}	13.4^{a}
Dry weight (g)	0.05^{c}	0.096^{c}	0.49^{b}	1.14^{a}	2.21^{a}

*Values in a row followed by the same letters indicate no significant differences between treatments at $P \leq 0.05$.

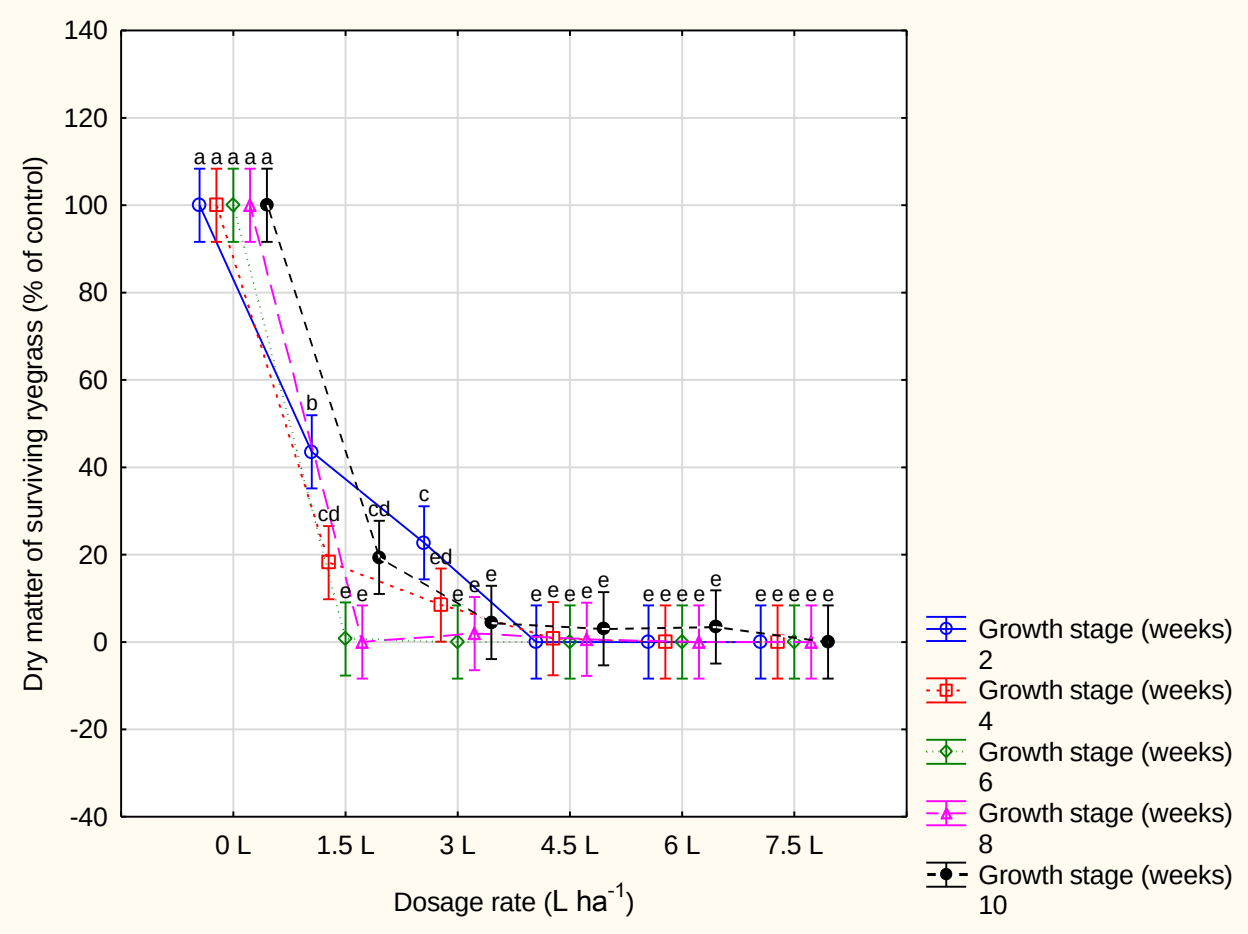

Figure 5.4: Dry matter of surviving plants as a percentage of the unsprayed control plants after application of various glufosinate ammonium dosage rates at five different growth stages for method 1. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

5.3.2 Field experiments

A. Welgevallen experimental farm

The ANOVA table (see Appendix 9) showed that the plant age and herbicide dosage rates had no significant interaction (P-value= 0.077). **Table 5.5** showed that generally, higher dosage rates gave better control of ryegrass. The only dosage rate which achieved mortality rate of over 80% was the 10 L ha^{-1} treatment. A relative higher control percentage, ranging between 60 and 80% was recorded for dosage rate of 5 and 7.5 L ha^{-1}. **Table 5.6** showed an inexplicable decrease in control at 6-week old ryegrass to 34.15%. However, control at 2-, 4-, 8- and 10-week old ryegrass did not differ significantly. Their mortality rates ranged from approximately 47 to 55%.

Table 5.4: Ryegrass percentage mortality against five glufosinate ammonium dosage rates on Welgevallen experimental farm

Dosage rate (L ha^{-1})	0	2.5	5	7.5	10
Mortality rate (%)	0.00000^{e}	10.700^{d}	61.700^{c}	75.250^{b}	89.550^{a}

Table 5.5: Ryegrass percentage mortality against five growth stages in weeks on Welgevallen experimental farm

Growth stage (weeks)	2	4	6	8	10
Mortality rate (%)	49.100^{ab}	51.950^{ab}	34.150^{c}	47.050^{b}	54.950^{a}

B. Roodebloem experimental farm

The ANOVA table of Roodebloem farm showed that there was an interaction between plant growth stage and glufosinate ammonium dosage rates (P-value ≤ 0.0001) (see Appendix 10). The interaction graph (**Figure 5.5**) showed a unique trend of glufosinate ammonium when sprayed at a dosage of 2.5 L ha^{-1}. There was almost 95% control of ryegrass at week 2 and a drastic decrease in control at week 4 to a value not significantly different from 0%. The control at 2.5 L ha^{-1} remained constant for 4-, 6- and 8-week old ryegrass with a slight increase to 20% control at week 10. Glufosinate ammonium dosage rate of 5 L ha^{-1} controlled ryegrass at 100% for week 2 but decreased with 80 to 85% at week 4 and 6. Another decrease in percentage mortality was observed at week 10 to 70%. At 7.5 L ha^{-1} dosage rate glufosinate ammonium showed a constant control ranging between 80 and 100% for all growth stages. A dissimilar trend observed for glufosinate dosage rate of 10 L ha^{-1}. At week 2, control was 80% and increased gradually with the increasing number of weeks reaching a control of 100% at week 6. A gradual decrease in mortality was noticed from week 6 up to week 10 where the percentage mortality reduced to 70%.

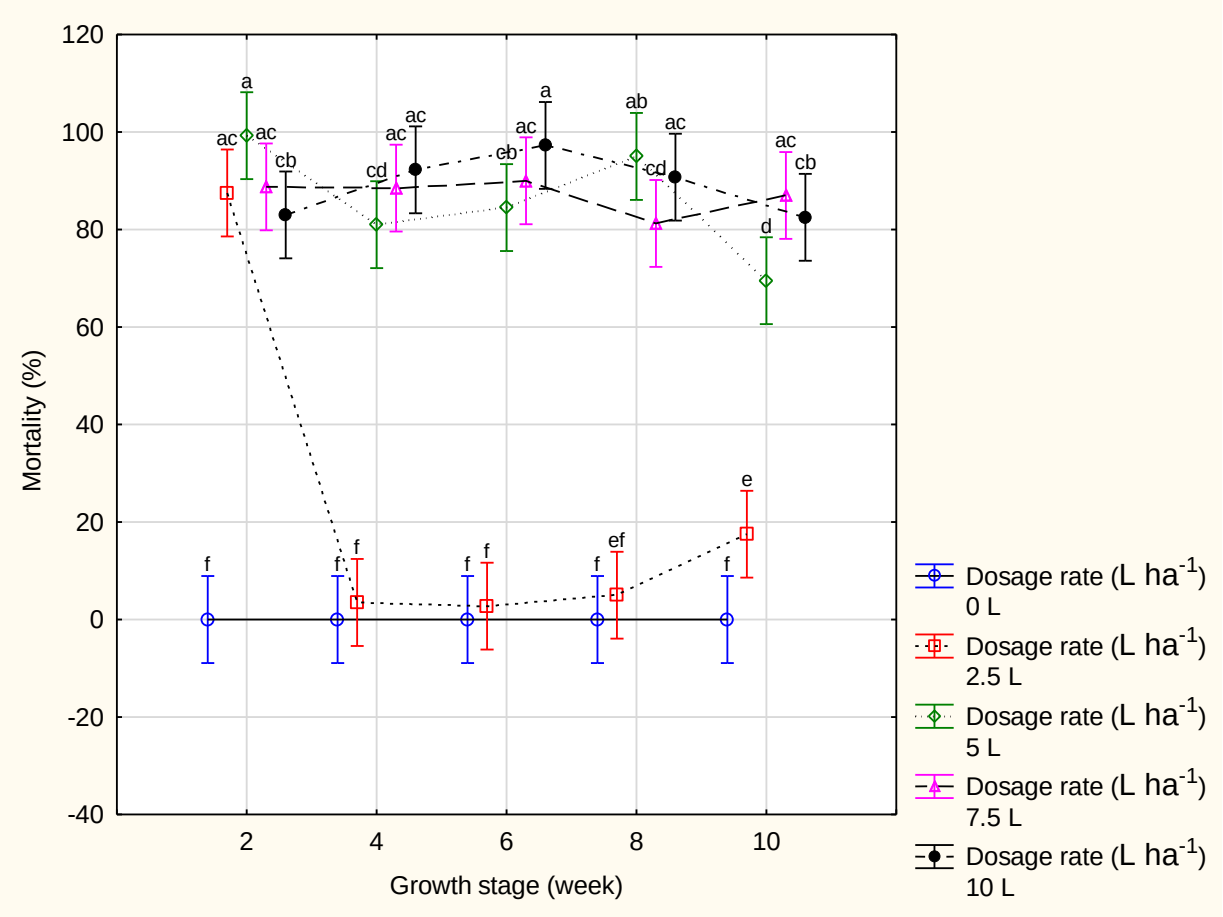

Figure 5.5: Mortality rates of ryegrass after application of various glufosinate ammonium dosage rates on Roodebloem experimental farm at five different growth stages. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

C. Langgewens experimental farm

The ANOVA table of Langgewens farm showed that there was an interaction between plant growth stage and glufosinate ammonium dosage rates (P-value ≤ 0.0001) (see Appendix 11). Glufosinate ammonium dosage rates of 2.5, 5, 7.5 and 10 L ha^{-1} had a general trend which showed an increase in control of ryegrass at week 4 shifting to a decrease in control at week 6 then a gradual increase of percentage mortality at week 8 (**Figure 5.6**). A drastic increase of percentage mortality was observed across all glufosinate dosage rates at 10-week old ryegrass. Glufosinate dosage rates of 2.5 L ha^{-1} gave a slightly dissimilar trend which showed a decrease in ryegrass control as plant age increased. However, there were no significant differences between weeks in the means of mortality at 2.5 L ha^{-1}except for 100% control at 10-week old ryegrass. Glufosinate ammonium dosage rates of 5 L ha^{-1} also showed no

significant differences in the mortality means for different plant growth stages. Herbicide dosage rates of 7.5 and 10 L ha^{-1} showed statistically significant differences in percentage mortality as the plant age increased. There was 100% control of 10-week old ryegrass using all dosage rates. A probable cause could be the dry spell which was experienced in August and September.

Tables 5.6, 5.7 and 5.8 show that leaf number, leaf area and plant height of ryegrass controls growing at Roodebloem farm was lower compared to Langgewens and Welgevallen experimental farms. **Figure 5.7** showed how the influence of climatic condition and environment varied with different farms at week 10. At Langgewens experimental farm, ryegrass showed a more pronounced herbicide x environmental effect where most of the plants turned yellow. However, plants in control treatments (**Figure 5.8**) were not as severely affected. **Figure 5.9** showed typical results at of Langgewens 10-week old ryegrass at evaluation time for all blocks regardless of dosage rate applied. This could explain the high mortality observed at Langgewens experimental farm (**Figure 5.6**), where control drastically reached 100% for all dosage rates applied.

Table 5.6: Vegetative growth parameters of control ryegrass plants at different growth stages in Welgevallen experimental farm at the time of spraying

	WELGEVALLEN				
Week	**2**	**4**	**6**	**8**	**10**
Leaf number*	4.3^{c}	4.4^{c}	5.9^{cb}	7.7^{ab}	12.1^{a}
Plant height (cm)	9.85^{c}	13.5^{b}	12.1^{bc}	14.8^{b}	32.4^{a}
Leaf area (cm^2)	3.58^{c}	4.97^{bc}	4.64^{bc}	20.144^{b}	74.025^{a}
Fresh weight (g)	0.049^{c}	0.064^{bc}	0.1^{b}	0.52^{bc}	3.14^{a}
Dry weight (g)	0.011^{c}	0.05^{b}	0.06^{b}	0.24^{cb}	0.79^{a}

*Values in a row followed by the same letters indicate no significant differences between treatments at $P \leq 0.05$

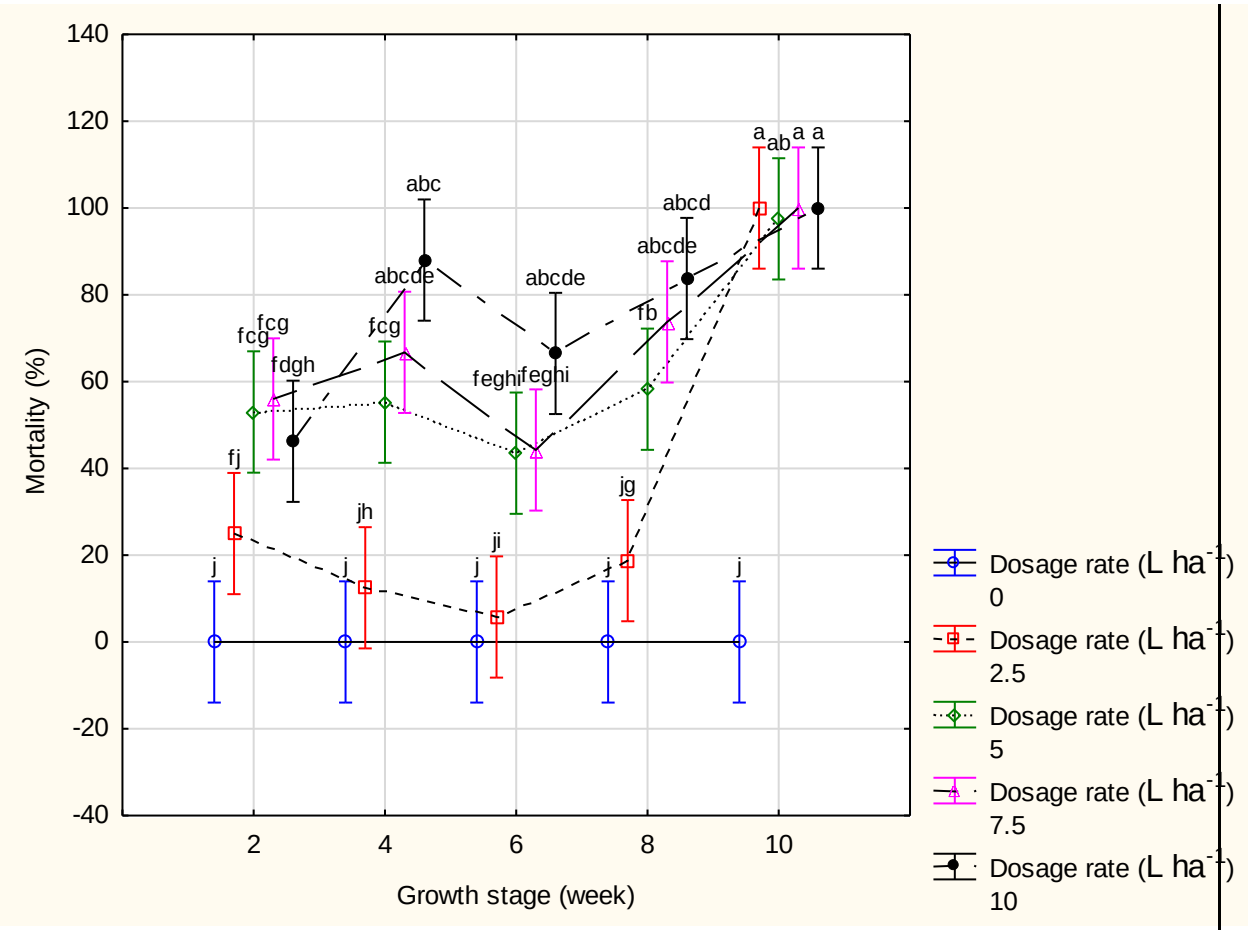

Figure 5.6: Mortality rates of ryegrass after application of various glufosinate ammonium dosage rates on Langgewens experimental at five different growth stages. Means indicated by different letters differed significantly at P ≤ 0.05 according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

Table 5.7: Vegetative growth parameters of control ryegrass plants at different growth stages in Roodebloem experimental farm at the time of spraying

	ROODEBLOEM				
Week	**2**	**4**	**6**	**8**	**10**
Leaf number*	3^{b}	6^{a}	6^{a}	6^{a}	9^{a}
Plant height (cm)	7.4^{d}	10.4^{c}	15.1^{b}	29.3^{a}	28.3^{a}
Leaf area (cm^2)	2^{e}	6.27^{d}	11.94^{c}	25.93^{b}	57.84^{a}
Fresh weight (g)	0.03^{d}	0.06^{c}	0.25^{b}	0.5^{b}	2^{a}
Dry weight (g)	0.017^{b}	0.045^{b}	0.052^{b}	0.254^{a}	0.516^{a}

*Values in a row followed by the same letters indicate no significant differences between treatments at P ≤ 0.05

Table 5.8: Vegetative growth parameters of control ryegrass plants at different growth stages in Langgewens experimental farm at the time of spraying

	LANGGEWENNS				
Week	**2**	**4**	**6**	**8**	**10**
Leaf number *	3.5^{c}	6.2^{b}	6.2^{b}	7.3^{b}	14.4^{a}
Plant height (cm)	9.6^{c}	11.4^{c}	17.8^{b}	18.1^{b}	44.1^{a}
Leaf area (cm^2)	3.98^{c}	7.46^{c}	11.7^{bc}	16.68^{b}	126.19^{a}
Fresh weight (g)	0.033^{c}	0.073^{c}	0.253^{bc}	0.485^{b}	5.723^{a}
Dry weight (g)	0.022^{c}	0.052^{c}	0.11^{bc}	0.273^{b}	1.779^{a}

*Values in a row followed by the same letters indicate no significant differences between treatments at $P \leq 0.05$

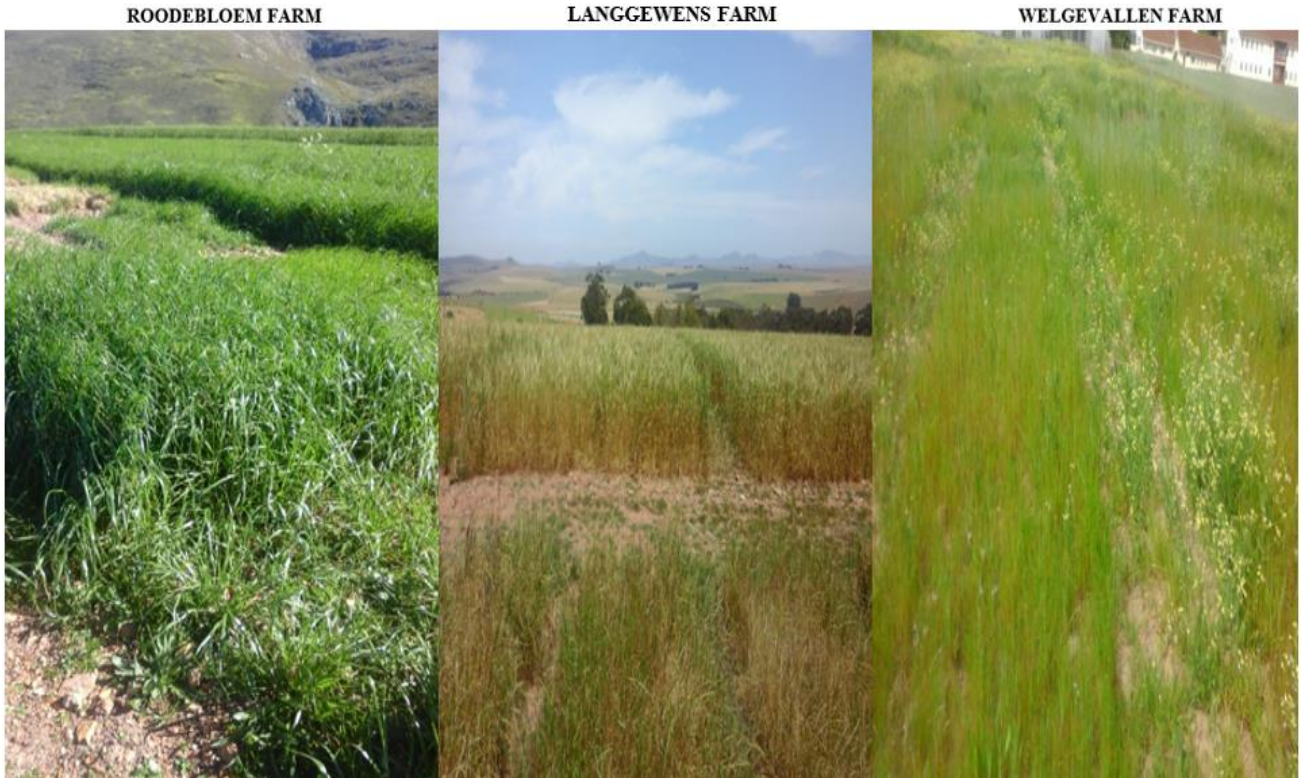

Figure 5.7: Roodebloem, Langgewens and Welgevallen experimental farms at 10 weeks.

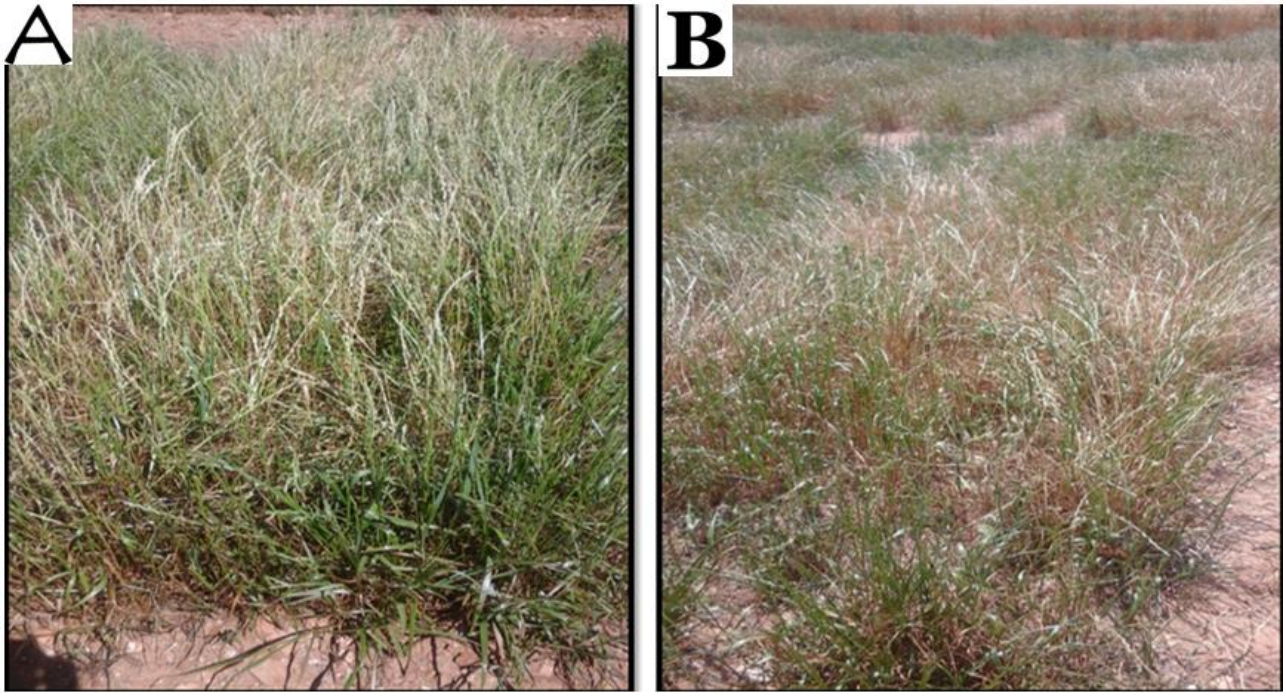

Figure 5.8: Comparison of 10-week old control ryegrass at the time of spray (A) and evaluation time (B) at Langgewens experimental farm

Figure 5.9: Typical results obtained at evaluation time for 10-week old plants regardless of dosage rate at Langgewens experimental farm.

Table 5.9: Rainfall for Roodebloem experimental farm during the year 2015

DATE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1						9,4			2,3		14,4	
2						63,8						
3		0,6			0,1	28,1						
4				9,1		4,8		1,1				
5		1,7	0,2	0,5	0,3		0,1					
6		0,1						0,1				
7											0,3	
8								0,1	0,1			
9					0,7		0,7	0,2				
10	0,1	0,7			0,7				2,3	1,6		
11							4,8		2,8		0,3	
12		2,1		0,1			6,5	0,4				
13		3,1			2,3		2,1	1,2		0,1		
14	0,2	6,8		1,5	2,9					8,6		
15		0,5		7,2						2,2		
16	15		9,8	0,9	1,6	13,6		2,7	1,7			
17			1,2			4,6	17,1	1,1	1,3			
18							4	2,1	2			
19		1,1						0,2				
20						0,1	3,4				8,9	
21		1,9	1,1				0,6			1,2	14,5	
22				0,2		0,3	4,8					
23			0,4			0,7	15,7					
24		0,7	0,8			13,4	16					
25			0,2			0,5	1,8	5,1				
26	1,2		1						6,1		3,6	
27	0,4		11,3					0,3	20,4			
28												
29				0,1		0,4		2	0,1			
30				0,1		0,1	13,2	9,5	28,1	1,7		
31	0,6				3,9		0,4	27,9		2,3		
TOTAL	17,5	19,3	26	19,7	12,5	139,8	91,2	54	67,2	17,7	42	0
									TOTAL RAINFALL			506,9

(blank) indicates that no rain fell on that day , ***/--- indicates that data is missing or not yet available in the current month

Table 5.10: Rainfall for Langgewens experimental farm during the year 2015

DATE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1											3	
2						2,5					11	
3						16						
4						8,5		3				
5								2				
6												
7									1			
8									1			
9												
10												
11							8,5					
12							1					
13												
14					6,5			12				
15									0,5			
16	7				3,5	14			0,5			
17						14	1,5					
18							18					
19												
20											2	
21			2,5								6	
22									5			
23			7,5				4					
24						7	11					
25						4		5				
26												
27												
28												
29												
30					6		5,5		2			
31					1,5		8	6				
TOTAL	7	0	10	0	17,5	66	57,5	28	10	0	22	0
									TOTAL RAINFALL 2015:			218

(blank) indicates that no rain fell on that day , ***/--- indicates that data is missing or not yet available in the current month

Table 5.11: Rainfall for Welgevallen experimental farm during the year 2015

DATE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1											5	
2						11,8						
3						44		11				
4		2						5				
5	0,1				3							
6									3			
7									6,5			
8											0,7	
9		4							15	3		
10									3,5			
11							10,5					22
12							3,5	1,5				
13		0,3			7,5			10		1		
14				0,5		A			1,5			
15	14,5				3,2	19.5 C		0,2	3,5			7
16						20	3					
17							35,5					
18												
19											4	
20			2								7	
21												2
22							15					
23						24	21	0,5				2,5
24						5,5		7,5				0,2
25					1,5							
26					6,8				3			
27						0,9						
28				2		1,5						
29		***		0,3	11		33	A	4,5			
30	1,8	***					2	9.5 C				
31		***		***		***		1,5	***	4,5	***	
TOTAL	16,4	6,3	2	2,8	33	107,7	123,5	37,2	40,5	8,5	16,7	33,7
									TOTAL RAINFALL			428,3

(blank) indicates that no rain fell on that day , ***/--- indicates that data is missing or not yet available in the current month

Table 5.12: Wind speed at Cape Town International recorded at 2 pm during the year 2015

DATE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	4.9	12.2	12.6	7.9	1.7	10.8	5.3	1.6	9.5	3.1	10.2	4.1
2	5.7	5.3	5.8	11.8	4	9.1	4.7	3.5	9	8.8	9.8	7
3	6.3	6	4.2	12.8	5.3	4.1	2.7	11.3	1.7	7.2	8	4.4
4	9.5	3.2	5.3	11	3.7	8	2.7	8.7	3.1	7	5	5.9
5	7	4.3	9.3	7	6.4	5	8.4	4.8	0	5.5	7.7	5.8
6	9.8	6.3	6.4	4.5	8.5	3.6	3.3	2.1	8.9	4.2	9.7	3.7
7	5.3	5.7	10	8.2	3.9	2.1	4.6	3.6	5.4	7.8	6.4	10.7
8	9.9	11.1	9.6	5.4	3.7	3	1.5	3.6	5.8	5.6	8.2	8
9	8.9	9.4	3.7	3.8	9.2	3.3	2.5	7.1	6.4	4	6.6	6.3
10	11.4	10.4	10.3	6.1	5.2	3.5	1.5	5.6	6.4	7.2	11.2	5.6
11	6.6	9.9	5.8	3.2	7	1.3	6.2	2.8	4	4.4	8.4	7.7
12	5.6	6.6	6.3	4.9	2.9	1.5	6.3	2.8	10	5.6	7.6	8.4
13	10.2	5.4	12.2	2.1	6.2	7.4	3.6	8.1	3.8	8.5	8.7	7.8
14	5.7	9.5	5.2	6.7	3.9	9.9	1.9	2.7	8.6	9	5.4	8.7
15	13	11.5	5	5.7	6.2	4.9	4	7.2	1.8	4.2	8.5	7.5
16	6.3	4.3	12.7	8.3	1.8	7.2	5.4	7.8	8.1	4	5.2	5
17	4.9	3.8	6.2	2.1	1.7	2.8	8.1	7.4	10.7	5.4	5.5	4.4
18	10.6	9	5.3	4.5	7.6	4.7	3.2	9.5	10.8	7.9	6.4	4.9
19	11.7	9.9	4	6.1	6	4.3	4.3	8.5	2.7	3.7	8.4	9.6
20	4.9	10	10.2	6.4	2.4	3.3	10.1	3.8	6.2	5.3	8.5	5.7
21	6.6	10.8	10.3	4.5	3.1	4.7	13.3	3.2	8	11.4	7.3	7.2
22	6.2	4.7	8.1	10.8	1.9	8	8.8	5.3	5.7	9.3	3.2	8.1
23	7	6.9	6.9	5.7	4.9	7.7	5.9	4.3	7.4	6	10.2	6.7
24	7.8	10.8	9	2.2	5.4	7	4.6	6.1	10.1	10.1	6.4	7.2
25	7.6	8.5	6.8	5.7	8	3.2	9	6.2	5.5	5.3	9.8	10.4
26	2.8	8.6	3.5	7.8	6.2	5.2	1.6	7	10.8	7.4	11.9	10.1
27	13.1	11.6	7.7	4.3	5.5	2.9	5.6	2.5	7.4	***	5.6	11.3
28	6.6	7.9	4.4	7.6	9.2	5.2	7.5	7.4	4.6	8	5.5	10
29	5.4	***	6.1	6.2	7.2	2.6	11.9	7.4	9.5	11.5	6.9	7.3
30	10.4	***	5.3	2.1	5.3	4	6.5	5.7	9.6	11.5	3.7	3.9
31	6.6	***	5.2	***	7	***	5.5	8.6	***	5.4	***	7.6
average	7.7	8	7.2	6.2	5.2	5	5.5	5.7	6.7	6.8=	7.5	7.1

(blank) indicates that no rain fell on that day , ***/--- indicates that data is missing or not yet available in the current month

5.4 DISCUSSION

Glasshouse experiment

At 4.5 L ha^{-1} dosage rates and higher, method 1 proved that ryegrass growth stage has no significant effect on control by glufosinate ammonium except when plants are 10-weeks old. The results showed that there was an increase in dry weight accumulation for 10-week old ryegrass, however, leaf number and leaf area were lower compared to the rest of the growth stages indicating a high proportion of stem material. It can be deduced that the metabolism and growth rate of ryegrass at this stage was lower, reducing glufosinate ammonium activity after absorption was also less effective. Low efficacy in control of 10-week old ryegrass can be ascribed to this reason since glufosinate ammonium action depends entirely on enzymatic reaction (Everman et al. 2009; Avila-Garcia and Mallory-Smith 2011). According to the relation between plant metabolism rate and herbicide efficacy, it would be expected that control of young plants such as 2- and 4-week old ryegrass would be higher. This study in contrary showed poor control of young plants. Steckel et al. (1997a) highlighted that smaller plant leaf area reduces efficacy of herbicides by reducing interception of herbicide by foliage. Plant height, leaf number and leaf area at 2- and 4-weeks were small and could have contributed to poor control as compared to week 6 and 8.

No significant differences of ryegrass mortality between growth stages was shown in method 2 using a dosage rate of 3 L ha^{-1} and above, except for 8- and 10-week old plants using a dosage of 4.5 Lha^{-1}. Glufosinate ammonium mode of action inhibits glutamine synthetase from converting glutamate to glutamine which results in accumulation of ammonia, a process that will affect actively photosynthesizing plants regardless of their growth stage (Steckel et al. 1997a; Sellers et al. 2004; Manderscheid et al. 2005; Avila-Garcia and Mallory-Smith 2011). The proportion of glutamine synthetase vary between plants depending on their photosynthetic type and environmental growth conditions (McNally et al. 1983). Given that ryegrass plants were exposed to the same environmental conditions during the present study, and because photosynthetic type was similar, proportions of glutamine synthetase that glufosinate ammonium needed to inhibit were probably about the same, which could explain the lack of differences in control between the different growth stages of ryegrass.

Both methods produced a general similar trend in the control of ryegrass when using 3 L ha^{-1} dosage rates and above. However, there were inconsistences in the controlling of 6- and

8-week old ryegrass between the two-separate analyses. For 6-week old ryegrass, the different mortality percentages were not significantly different for both methods. A most probable cause for the different mortality rate for 8-weeks old ryegrass would be spraying at different times since ryegrass was planted once. Different climatic environments in terms of light conditions such as day length or cloud cover after spraying cannot be completely avoided. However, the two methods did not differ much regardless of the differences shown by 6- and 8-week old ryegrass.

Field experiments

There was no significant difference in the control of different sized ryegrass on Welgevallen experimental farm. However, a noticeable decrease in mortality was observed at week 6. A possible explanation for the decrease in control would be weather conditions at the spray date. Wind speed at the time of spray was 7.5 km/hr which is significantly high. Drift in herbicide during application might have resulted in less absorption of glufosinate ammonium. Glufosinate ammonium is a foliar active herbicide that for optimal efficacy depends on the environmental conditions during and after spraying (Everman 2008). This decrease was not observed in Langgewens and Roodebloem experimental farm.

Roodebloem experimental farm observations showed no significant difference in control of ryegrass regardless of the herbicide dosage rate applied and ryegrass growth stage. An interesting trend was shown by 2.5 L ha^{-1} dosage rate where control of 2 weeks old ryegrass was exceptionally high as compared to the rest of the growth stages. This finding supports other studies which found that control of younger weeds is higher than that of mature weeds due to their susceptibility (Kells et al. 1984; Chauhan and Abugho 2012). Average leaf number, area and plant height at week 2 for Roodebloem experimental farm was lower than that of Langgewens and Welgevallen experimental farm. This might explain the unexpected mortality of approximately 90% ryegrass control using a relatively low dosage rate of 2.5 L ha^{-1}. Another probable cause of these unexpected results would be an unidentified experimental error.

Inconclusive results were obtained for ryegrass control with glufosinate ammonium on Langgewens experimental farm. Control of ryegrass was generally poor when compared to observations made at Roodebloem and Welgevallen experimental farms. Even a high dosage rate of 10 L ha^{-1} controlled only 50% of 2-week old ryegrass. The possible cause for this trend was a high soil seedbank of wild ryegrass at Langgewens farm. There was emergence

of ryegrass about a week after spraying. Time of application of herbicide during the season must be an important consideration to ensure that most of the dormant seed in the seedbank would have germinated at the time of spraying. The shift to 100% control at week 10 at all glufosinate ammonium dosage rates was an anomaly. The most influential factor for such a swing probably was the total absence of rain during the time of spraying and thereafter. Hence, mortality of ryegrass at week 10 was not only accountable to glufosinate ammonium only but likely also the dry spell.

When comparing the effect of growth stage and dosage rate on all the three farms, the overall finding was that there were no significant differences in controlling ryegrass at different growth stages. Also, glufosinate ammonium dosage rates did not show significant differences in controlling ryegrass except for 2.5 L ha^{-1} which resulted in control of less than 20%. Another observation in the field experiments was that every farm gave a unique trend and this trend could be due to soil or environmental factors. This shows the importance of gathering all facts for a specified region before application of glufosinate ammonium (Duke and Cerdeira 2005; Everman 2008). For this experiment, ryegrass soil seedbank and rainfall occurrence were important factors that needed to be considered before glufosinate ammonium application.

Comparison of glasshouse and field experiments

Controlling different stages of ryegrass using a specific glufosinate ammonium dosage rate in the glasshouse was not significantly different and this was also true for the field experiments. However, control of ryegrass even with 10 L ha^{-1} in the field never gave complete control of 100%. A 100% control level was observed for lower dosage rates of 4.5 and 6 L ha^{-1} in glasshouse experiments. A possible explanation for these differences was the different environment in which ryegrass was growing. Adverse climatic conditions in the field could have resulted in poor absorption of glufosinate ammonium. McNally et al. (1983) also noted that the environment causes only a proportion of glutamine synthetase to be inhibited in plants, hence, probably accounting for the difference in control of glasshouse and field ryegrass in the present study.

Another possible cause for different results obtained in the glasshouse and field could be the effect of the volume of spray water. Glufosinate ammonium was applied in 200 L of water per hectare for both glasshouse and field experiments. A study done by Cody et al. (2015) concluded that contact herbicides require higher spray volumes to increase efficacy.

Maybe 200 L water per ha was enough for the plants in pots because they were only 4 per pot but in the field the plants were growing much more densely, therefore, 200 L water might not have been enough to get good coverage of all the leaves.

Water quality could have been another reason why there were different results between glasshouse and field experiments. Deionised water was used for glasshouse experiments while tap water was used for field experiments. The quality of tap water was probably poor as compared to deionised water (generally pH values of higher than 7,5 occurred). Poor quality reduces efficacy of glufosinate ammonium (Bayer Crop Science n.d.). However, adjuvants can be added to spray water as activator agents which increase herbicide activity. Addition of AMS to spray water can increase efficacy of glufosinate ammonium as reported in Chapter 4.

5.5 CONCLUSION

Growth stage of ryegrass did not have a significant influence on efficacy of glufosinate ammonium in both field and glasshouse experiments. The major difference between field and glasshouse experiment was that control of ryegrass in the field rarely reached 90%, even at the high dosage rate of 10 L ha^{-1} glufosinate ammonium, whereas control of ryegrass in a glasshouse reached 100% with a low dosage rate of 4.5 L ha^{-1}. Reported results in Chapter 4 suggest that spray water quality and efficacy of glufosinate ammonium can be enhanced by adding AMS.

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CHAPTER 6

EFFICACY OF GLUFOSINATE AMMONIUM WITH ADDED ADJUVANTS ON DIFFERENT GROWTH STAGES OF RYEGRASS

ABSTRACT

A glasshouse and field experiment was conducted at Welgevallen experimental farm to investigate the efficacy of glufosinate ammonium that has added adjuvants on different growth stages of ryegrass plants (*Lolium multiflorum* cv Energa). In the glasshouse, glufosinate ammonium was applied in mixtures with 2% ammonium sulphate (AMS) (Velocity®), 0.5% nitrogen solution/non-ionic surfactant (Summit Super®), and without an adjuvant. Glufosinate ammonium rates used in the glasshouse were 0, 1.5, 3, 4.5, 6 and 7.5 L ha^{-1}. Three different growth stages of ryegrass (3, 6 and 9 weeks) were obtained by planting the ryegrass at 3-week intervals from the date the experiment was initiated. Control of 6-week old ryegrass was more effective regardless of the adjuvant used. Glufosinate ammonium with added AMS controlled ryegrass more effectively than glufosinate ammonium applied alone or with added nitrogen solution/non-ionic surfactant. A similar experiment was done in the field using glufosinate ammonium at 0, 2.5, 5 and 7.5 L ha^{-1} dosage rates and two wild ryegrass growth stages (young and mature). Young ryegrass had an average leaf number of 6 and mature ryegrass had 15 leaves. Mixtures with glufosinate ammonium were 2% ammonium sulphate (AMS), a methylated vegetable oil with alcohol ethoxylate (Ballista®) and without an adjuvant. Results showed that glufosinate ammonium with added AMS controlled young ryegrass better than glufosinate ammonium alone or with added Ballista®. There was no significant difference in glufosinate ammonium control of mature ryegrass with or without added adjuvants. A dosage rate of 7.5 L ha^{-1} controlled 80 to 100% ryegrass regardless of growth stage and adjuvant.

Keywords: adjuvant, ammonium sulphate, glufosinate ammonium, methylated vegetable oil, nitrogen solution/non-ionic surfactant, ryegrass growth stage.

6.1 INTRODUCTION

Competition of weeds with crops result in significant yield loss if their control is delayed (Rosales-Robels et al. 1999). A management strategy best suitable for weeds is to control them before they cause significant yield loss and become more tolerant to herbicides.

Herbicide resistance in ryegrass (*Lolium* spp.) has become a chief concern in South Africa (ARC Annual Report 2013/2014). However, the introduction of herbicide-resistant crops has paved the way for better control opportunities with non-selective herbicides such as glufosinate ammonium (Green and Owen 2011). Because glufosinate ammonium is a post emergent herbicide, its efficacy is greatly influenced by leaf age and developmental stage of weeds

Applying herbicides to weeds in their early growth stages has proved to reduce the application rates by 75% while effectively controlling the weeds (Ahmadi et al. 1980; Bellinder et al. 2003). Glufosinate ammonium is effective in controlling weeds, however, inconsistencies with different weeds species have been proven in field studies, including ryegrass (Pratt et al. 2003; Molefe 2015). Adjuvants negate reduced efficacy caused by adverse environmental and plant conditions, hence, they can be added to glufosinate ammonium in situations where control is expected to be lower than is acceptable (Martinson et al. 2002; Bellinder et al. 2003; Pratt et al. 2009).

Ammonium sulphate has been founded to increase efficacy of glufosinate ammonium (Pratt et al. 2009). Western Cape farmers have also been reported to use methylated vegetable oil (Ballista®) as an adjuvant to increase glufosinate ammonium efficacy. A combination of timely application of glufosinate ammonium together with the use of adjuvants might eliminate inconsistent weed control shown by glufosinate ammonium. Therefore, the objectives of this study were (i) to determine the efficacy of glufosinate ammonium as influenced by different ryegrass growth stages and (ii) to evaluate the effect of adjuvants ammonium sulphate (Velocity®), nitrogen solution (Summit Super®) and methylated vegetable oil (Ballista®) on efficacy of glufosinate ammonium on ryegrass under glasshouse and field conditions.

6.2 MATERIALS AND METHOD

6.2.1 Glasshouse experiment

The experiment was conducted in a glasshouse at Stellenbosch University Welgevallen experimental farm. Commercial ryegrass (*Lolium multiflorum* cv Energa) was grown in pots. The study was conducted in a glasshouse at 18/23℃ night/day temperatures. The design was a 3×3×6 factorial arranged in a randomized complete block design with 5 replications. The experimental factors were plant size at three levels (3, 6 and 9 weeks), adjuvant at three levels

(no adjuvant, nitrogen solution/non-ionic surfactant (Super Summit®) and ammonium sulphate (Velocity®) and glufosinate ammonium dosages at five levels (0, 1.5, 3, 4.5, 6 and 7.5 L ha^{-1}). Ryegrass was planted at 3-week intervals from the beginning of the experiment until the 9th week and all the pots were sprayed simultaneously after 12 weeks. The herbicide was applied on the 6th of April 2016 by means of a pneumatic pot sprayer at a pressure of 2 bar in 200 L ha^{-1} of water. After spraying, ryegrass was left overnight inside the spraying room to prevent the herbicide from being washed off by the automatic irrigation system. Evaluation was done 4 weeks after spraying

Seedlings were transplanted into 8 x 8 cm square plastic pots one week after being sown in 2 L plastic containers filled with potting sand. A nutrient solution was used to fertilize ryegrass plants during the course of the study. The composition is shown **Table 6.1**. Additional weeds were removed by hand. No pests and diseases were experienced in the glasshouse. An automated irrigation system was used to water the plants. The plants were irrigated at 8:00 am, 12:00 pm, 2:00 pm and 4:00 pm. The quantity of water per irrigation was adjusted depending on the plant growth stage to compensate for water loss.

Table 6.1: Composition of the nutrient solution used to fertilize the plants growing in pots

EC = 2.0			
Element (Macro)	Concentration mg L^{-1}	Fertilizer	Concentration g 1000L^{-1}
K^+	237.7	KNO_3	303
Ca^{++}	180	K_2SO_4	261
Mg^{++}	48.6	$Ca(NO_3)_2.2H_2O$	900
NO_3^-	661.33	$MgSO_4.7H_2O$	492
H_2PO_4	116.4	KH_2PO_4	136
SO_4	390.4		
(Micro)	mg L^{-1}		
Fe	0.85	Libfer (Fe EDTA)	6.54
Mn	0.55	Manganese sulphate	2.23
Zn	0.30	Zinc sulphate	1.33
B	0.30	Solubor	1.46
Cu	0.05	Copper Sulphate	0.20
Mo	0.02	Sodium Molibdate	0.13

6.2.2 Field experiment

The study was conducted under field conditions at Stellenbosch University Welgevallen experimental farm. The design was a 2×3×4 factorial arranged in a randomized complete block with 4 replications. The experimental factors were plant size at two levels (mature and young ryegrass), adjuvant at three levels (no adjuvant, ammonium sulphate and a methylated vegetable oil with alcohol ethoxylate (Ballista®) and glufosinate ammonium dosage rates at

four levels (0, 2.5, 5 and 7.5 L ha^{-1}). A field previously planted with wheat had grown a dense cover of volunteer weedy ryegrass (*Lolium* spp.). The plot was then used as experimental field after blocks and plots were demarcated hence no ryegrass was sown during the experiment. Controls were harvested at the time of spraying. Spraying was done when the ryegrass was young and again after 4 weeks from the first spray when the ryegrass was mature. Spraying dates were 24 May and 15 June 2016, respectively. Young ryegrass had an average leaf number per plant of 6 and mature ryegrass had 15 leaves per plant. A CP3 spraying knapsack was used to spray glufosinate ammonium in 200 L ha^{-1} of water.

6.2.3 Data collection

For both glasshouse and field experiments, one set of control plants was harvested at the time of spraying and the following variables were recorded:

a. Number of leaves per plant

The number of leaves per plant were counted and recorded. The mean number of leaves of the four plants was then used as the number of leaves per pot in the glasshouse experiment.

b. Leaf area (cm^2)

Leaf area per plant was measured using a leaf area meter (Li Cor) and the mean leaf area of the plants in a pot was then calculated.

c. Plant height (cm)

A calibrated ruler was used to measure plant height. The height considered was of the stems and leaves (above root) from the soil surface to the tip of the longest leaf of each plant. The mean plant height per pot was then calculated.

d. Wet biomass (g)

From each pot, plants were harvested at the soil surface by means of secateurs and put into paper bags and the wet biomass was then measured using an electronic balance and recorded. Wet biomass was expressed as biomass per pot.

e. Dry biomass (g)

After determining the wet biomass, the paper bags with plants were put into an oven and dried at 80ºC for 48 hours. The dry plants were then weighed on an electronic balance and the dry mass per pot was then calculated.

At four weeks after spraying the treated plants were evaluated for mortality and the dry mass of the surviving plants were recorded in the same way as described above. The variables were recorded as follows:

a. Percentage mortality (%)

Percentage mortality for both glasshouses and field experiments was recorded four weeks after spraying. Calculation for the glasshouse experiment was done using the following formulae;

Percentage mortality = $\frac{number\ of\ dead\ plants\ per\ pot}{4\ (plants\ per\ pot)} \times 100\%$

Percentage mortality in the field was determined by visual observation. Five individuals were invited to give estimated control percentages on specified sub-plots and the mean was calculated.

b. Dry matter of surviving ryegrass expressed as percentage of the control treatment

Dry matter of surviving ryegrass was calculated as percentage of the unsprayed control treatment on glasshouse plants. The plants were green and live at the time of evaluation. The calculation was done using the following formulae;

Dry matter of surviving ryegrass expressed as control percentage =

$$\frac{dry\ mass\ of\ of\ surviving\ plants\ per\ pot\ at\ evaluation(g\ )}{average\ live\ dry\ matter\ per\ pot\ (g) of\ control\ plants} \times 100\%$$

6.2.4 Data analysis

Data were subjected to an analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Bonferroni test for control variables recorded at spraying time and Fischer's $LSD_{0.05}$ for the data variables recorded at evaluation (four weeks after spraying). Bonferroni confidence intervals for differences of the means are wider than that of Fisher's LSD, therefore, Bonferroni test

was used for one-way ANOVA of control variables whilst Fischer's $LSD_{0.05}$ was used for the factorial ANOVA.

6.3 RESULTS

6.3.1 Glasshouse experiment

Analysis of variance for mortality showed a significant P-value of 0.002 for interaction of dosage rate, adjuvant and ryegrass growth stage (see ANOVA table in Appendix 12). The results of week 3 was variable (**Figure 6.1).** . Velocity® (AMS) significantly improved the control percentages at 1.5 and 3 L ha^{-1} dosage rates. Summit Super®, however, significantly reduced efficacy of glufosinate ammonium at the 4.5 L ha^{-1} rate. The control of 6-week old ryegrass ryegrass was better than the control of 3- and 9-week old ryegrass **(Figure 6.1)**. At 6 weeks, control with 3 L ha^{-1} was 100% with the two added adjuvants, whereas at the same dosage rate control with glufosinate ammonium alone was less than 100% but not significantly different from the other two treatments. For 3- and 9-week old ryegrass, Velocity® gave significantly better results as compared to Summit Super® and no adjuvant. A 100% control was reached with dosage rates of 4.5 L ha^{-1} using Velocity® at week 3 and only 1.5 L ha^{-1} was required to reach 100% control at week 9 with Velocity®. Control at 9 weeks was significantly better than control at 3 weeks. There was no significant difference in the action of glufosinate ammonium without an adjuvant and when using Summit Super® at 9 weeks.

There was no significant three-way interaction in percentage dry matter (P-value = 0.076). However, there were significant two-way interactions between growth stage and dosage rate (P-value = 0.0002) as well as growth stage and adjuvant (P-value = 0.01) (See ANOVA table in appendix 13). Control of ryegrass at 6 weeks was significantly greater than that at 3- and 9-week old ryegrass and significantly greater at 9 weeks than at 3 weeks regardless of the adjuvant added at a dosage rate of 3 L ha^{-1} **(Figure 6.2)**. At 6 weeks, the ryegrass required a dosage rate of 3 L ha^{-1} to give effectve control in terms of dry matter of surviving plants, while 3- and 9-week old ryegrass required a dosage rate of 4.5 L ha^{-1} for effective control. The treatment combination of 9 weeks and the adjuvant Velocity® gave the best control (dry matter of surviving plants) of 17% (**Figure 6.3)**. However, at 6 weeks the ryegrass was also controlled effectively with the dry matter of surviving plants ranging between 18 and 22% of the untreated control plants regardless of the adjuvant used. The use of the adjuvant Velocity® gave more consistent good results, with 20% or dry matter of live plants compared

to the untreated control. Glufosinate ammonium alone, and with the adjuvant Summit Super® added, showed poorer control of ryegrass than with Velocity® added except with 6 week old plants.

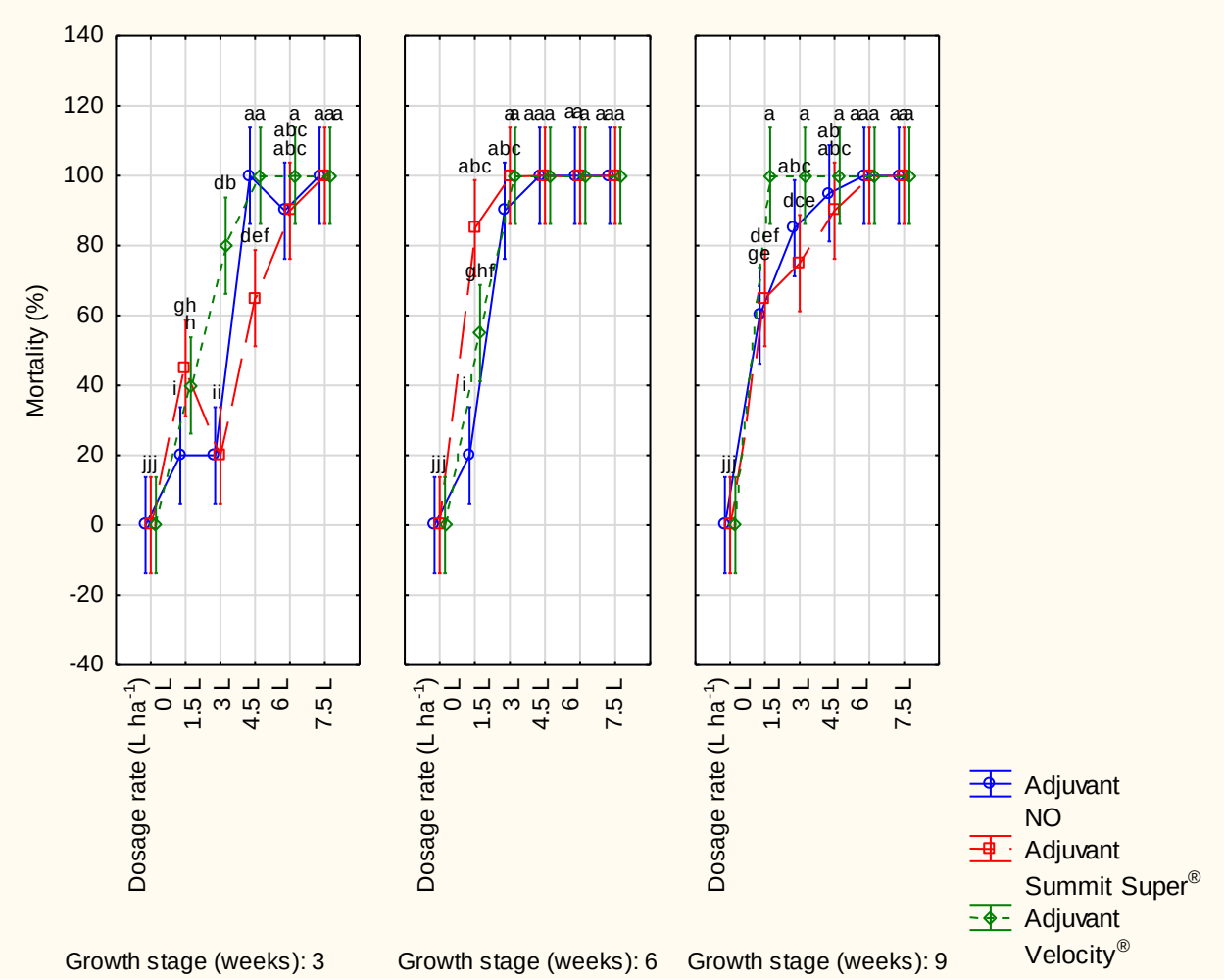

Figure 6.1: Mortality rates of ryegrass after application of various mixtures of adjuvants with glufosinate ammonium dosage rates at three different growth stages in the glasshouse. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

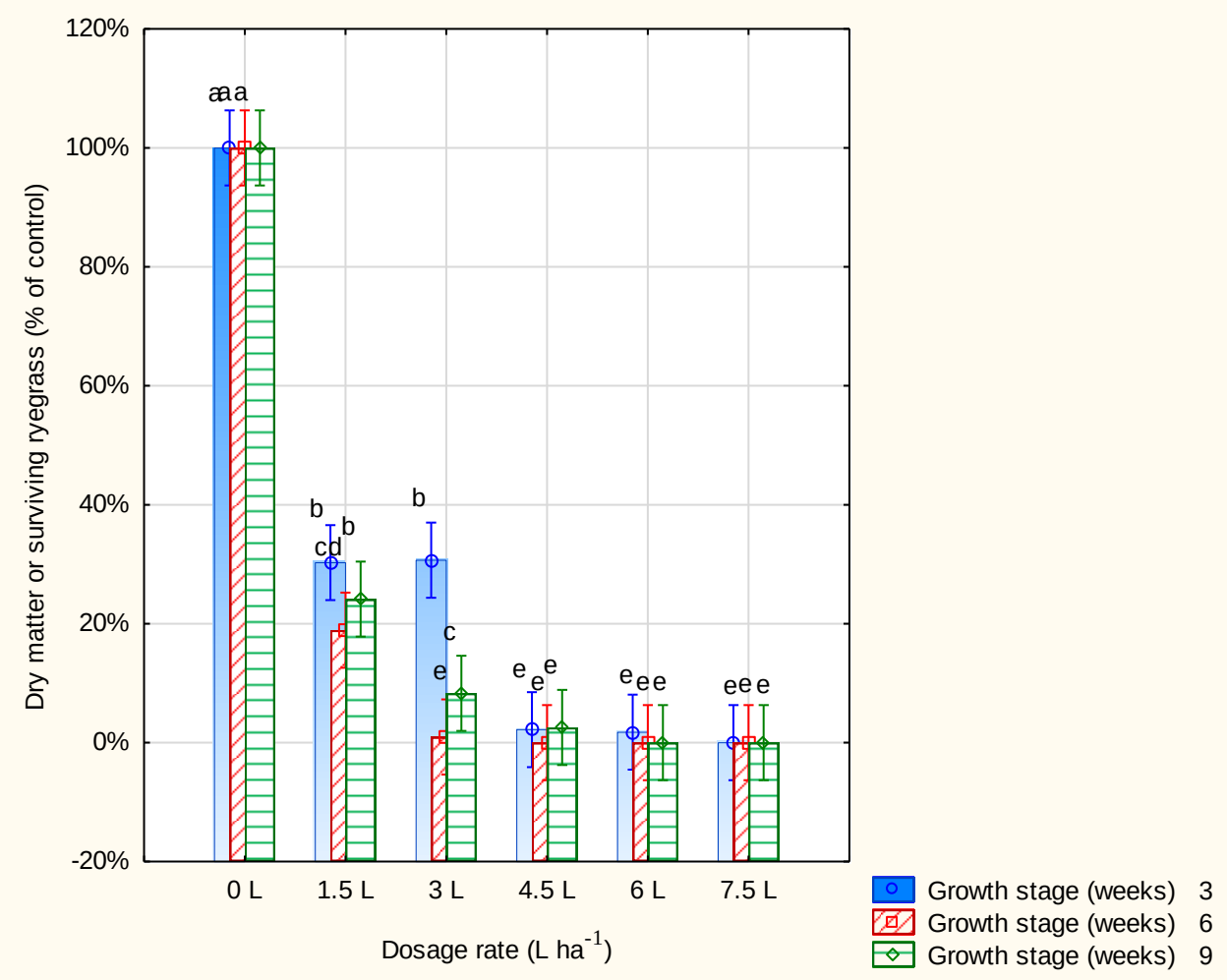

Figure 6.2: Interaction of glufosinate ammonium dosage rate and ryegrass growth stage on dry matter of surviving plants as a percentage of the unsprayed control ryegrass in the glasshouse. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

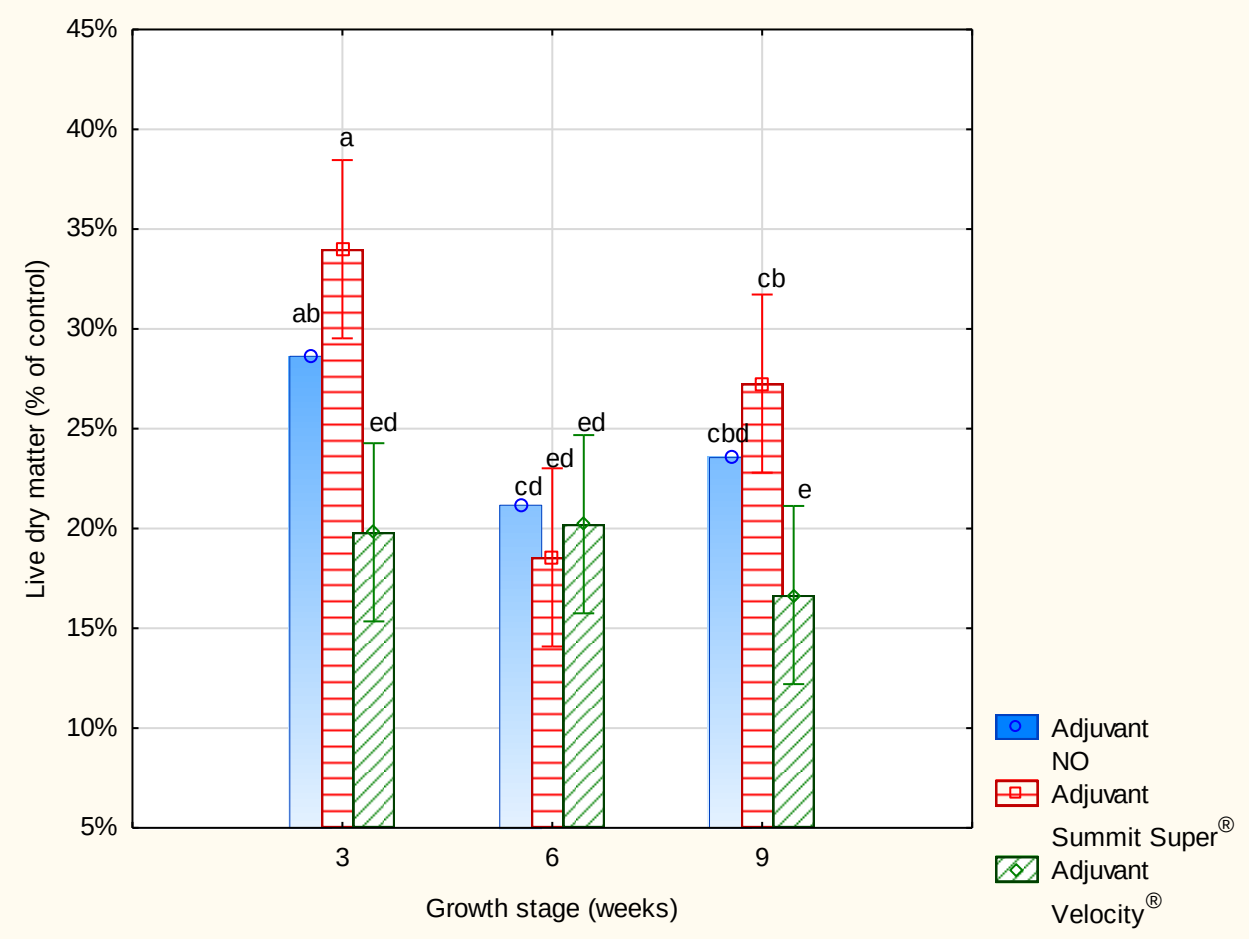

Figure 6.3: Interaction of adjuvant and ryegrass growth stage on dry matter of surviving plants as a percentage of unsprayed control ryegrass in the glasshouse. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

Table 6.2: Vegetative growth parameters of control ryegrass plants at different growth stages at the time of spraying in the glasshouse

	Growth stage		
Control variables	**3 weeks**	**6 weeks**	**9 weeks**
Leaf number*	7^{b}	16^{a}	19^{a}
Plant height (cm)	27.21^{b}	45.41^{a}	47.6^{a}
Leaf area (cm^2)	20.39^{b}	118.69^{a}	181^{a}
Fresh weight (g)	0.67^{b}	4.88^{a}	7.78^{a}
Dry weight (g)	0.04^{c}	0.69^{b}	1.66^{a}

*Values in a row followed by the same letters indicate no significant differences between treatments at $P \leq 0.05$.

6.3.2 Field experiment

The P-value from ANOVA of growth stage, adjuvant and dosage rate interaction was 0.382, and therefore, not significant (see Appendix 14). However, there was a significant two-way interaction between dosage rate and adjuvant (P-value= 0.032). Growth stage and dosage rate (P-value= 0.093), as well as growth stage and adjuvant (P-value= 0.284) interactions, were not significant. No significant difference was shown by adjuvants as different dosage rates were applied (**Figure 6.4**). The only significant difference was shown by a dosage rate of 2.5 L ha^{-1} where mortality of ryegrass was approximately 50% with addition of AMS as compared to 30 and 20% when using Ballista® and no adjuvant respectively. Dosage rates of 7.5 L ha^{-1} gave mortality percentage of over 80% regardless of the adjuvant used.

Mortality rates increased with an increase in dosage rates for both young and mature ryegrasses (**Figure 6.5**). Growth stage of ryegrass in the field has no significant effect on the efficacy of control, irrespective of dosage rates of glufosinate ammonium. The use of AMS adjuvant influenced the efficacy of glufosinate ammonium for young ryegrass only **(Figure 6.6**.) The influence of Ballista® on glufosinate ammonium efficacy on young ryegrass was not significantly different from treatments with no adjuvant. There was no significant difference in control of mature ryegrass when adjuvants were added as compared to no adjuvant. However, **Table 6.3** showed that percentage mortality of young and mature ryegrass was significantly different between the two growth stages.

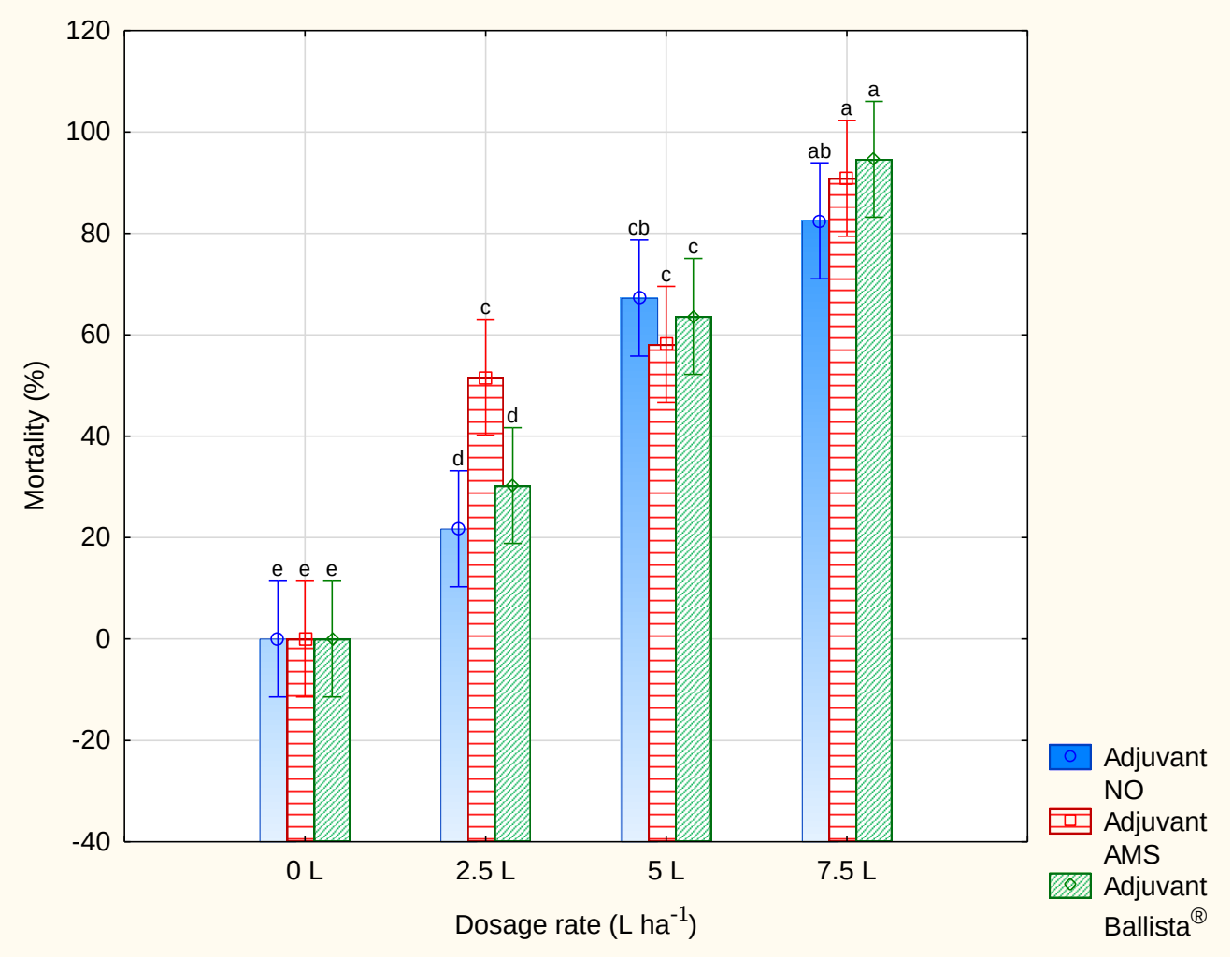

Figure 6.4: Interaction of dosage rate and adjuvant on percentage mortality of ryegrass in the field. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

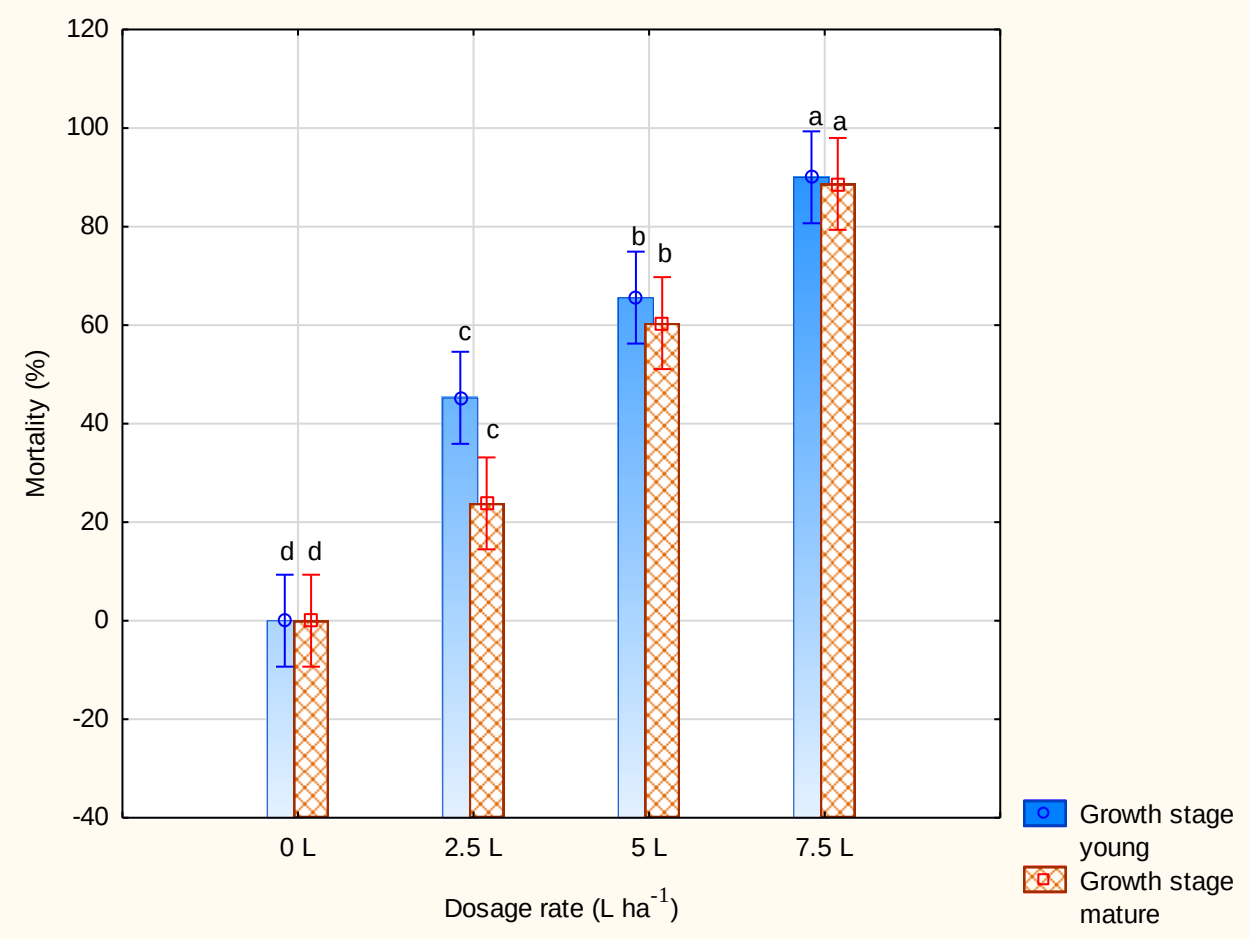

Figure 6.5: Interaction of dosage rate and ryegrass growth stage on mortality rate of ryegrass in the field. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

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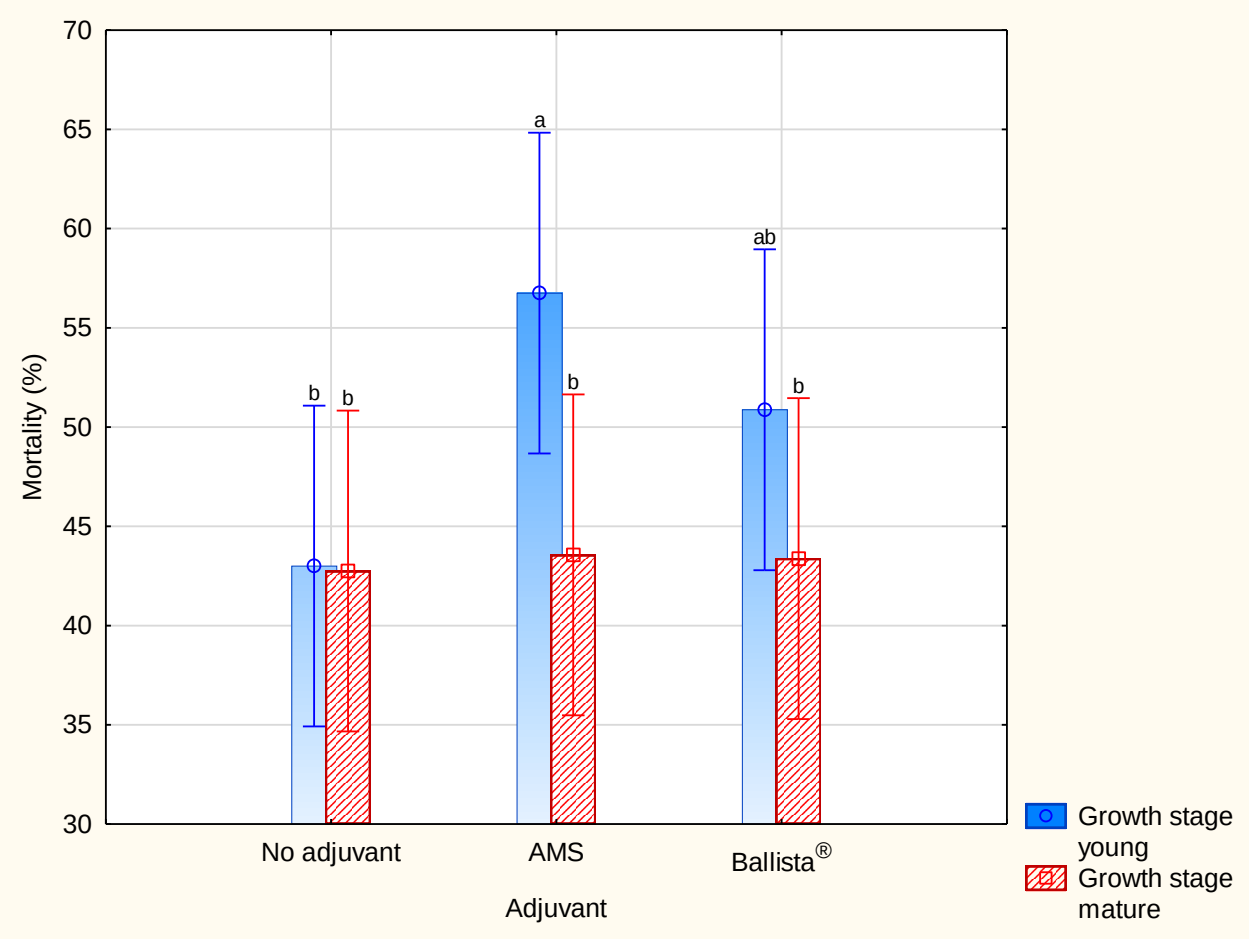

Figure 6.6: Interaction of adjuvant and ryegrass growth stage on percentage mortality of ryegrass in the field. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

Table 6.3: Percentage mortality of young and mature ryegrass in the field four weeks after spraying

	Growth stage	
	Young	**Mature**
Percentage mortality*	50.2^{a}	43.2^{b}

Values in a row followed by the same letters indicate no significant differences between treatments at $P \leq 0.05$.

Table 6.4: Rainfall for Welgevallen experimental farm during the year 2016

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
1				1	0,7		7		
2								22,5	
3									
4									4
5			0,8				14,5	2	23,5
6									
7									
8									3
9						27,5			
10									
11									
12					0,5			5	
13						4,5		8	
14		1,5				13	9,5	1,5	***
15						4,5	2		***
16									***
17									***
18			3,8		7				***
19		27				29,5	2,5	0,6	***
20			1,5	5,5		5,5	30		***
21				5			1	27	***
22				22			4,5	3	***
23					10		0,7		***
24	1,5			2					***
25			3	3			2		***
26			19,5			11	21		***
27						2	7		***
28				19,5			14,5		***
29			1						***
30		***	6,5			12			***
31		***		***		***		4	***
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Tot	1,5	28,5	36,1	58	18,2	109,5	116,2	73,6	30.5=

(blank) indicates that no rain fell on that day , *** indicates that data is missing or not yet available in the current month

Table 6.5: Temperature readings for Welgevallen experimental farm during the year 2016

Day	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
1	29,5	30,5	27,3	21,6	19	19,4	14,1	19,2	18,1
2	28,6	26,9	27,9	21,8	19,3	18,1	14,9	20,7	18,1
3	29,3	25,8	27,6	24,5	20,6	17,4	16,3	16,9	21,6
4	30,9	30,8	32,5	22,3	23	18,7	24,3	18,2	26,3
5	31,2	33,1	30,3	22,2	20,5	20,2	15,6	18,4	18,2
6	28,8	28,7	28,7	28,9	24,8	18,3	14,8	18,9	19,3
7	22,9	25,7	23,9	28,7	24,7	24,2	16,2	18	17,9
8	25,8	30,5	24,7	29,8	24,7	28,5	14,8	20,4	18,7
9	28	34,3	25	31,4	19,3	19,5	20,3	18,9	17,6
10	30	31,1	25,5	33,2	19,4	16,1	28,6	27,3	21,7
11	33,1	28,9	30	26,3	22,5	18,5	21,1	18,7	24,3
12	36,3	28,4	27,6	22,5	20,4	20,7	21,3	17,1	21,3
13	29,1	25,8	23,3	21,5	20,3	17,4	26,6	17,4	22,4
14	26,2	26,1	28,4	22,2	16,2	16,1	19,7	16,4	***
15	33,8	24,1	26,2	24,7	20,9	17,6	15,4	17,2	***
16	32,7	29,4	30,8	24,6	20,9	16,5	16,4	22,3	***
17	35,6	32,7	24,4	25,6	31,3	17,1	20,2	23	***
18	32,7	26,9	23,8	28,7	18,6	16,5	18,9	21,8	***
19	31,8	23,5	23,6	26,9	18,3	13,3	17,4	19,3	***
20	32,9	24,5	23,1	23,8	22	14,7	17	20,5	***
21	32,4	24,5	22,6	20,5	19,2	15,6	16,8	15,1	***
22	30,8	24,8	25,9	19,7	23,5	20,1	15,7	16,6	***
23	28,4	24,3	24,1	20,9	19,6	25,7	14,7	18,6	***
24	26,4	25,9	27,7	20,2	18,4	17,3	15,2	21,8	***
25	24,3	26,6	27,9	20,4	19,1	18,5	16,9	19,3	***
26	26,3	25,7	22,3	19,2	23,8	17,7	13,5	28,6	***
27	26,2	24,4	20,2	20,2	18,1	15,3	17	27,5	***
28	27,5	26,5	22,3	20,4	17,3	15	18	18,9	***
29	27,9	29,2	21,3	18,1	18,9	16	18,6	20,9	***
30	28,2	***	21,3	18,6	18,6	16,8	22,2	23,5	***
31	33,9	***	21,9	***	***	***	14,9	18,3	***
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Avg	29,7	27,6	25,6	23,6	20.8=	18,2	18	20	20.4=

Table 6.6: Vegetative growth parameters of control ryegrass plants at different growth stages at the time of spraying in the field

	Growth stage	
Control variables	**Young**	**Mature**
Leaf number*	6^{b}	15^{a}
Plant height (cm)	19.25^{b}	27.13^{a}
Leaf area (cm^2)	43^{b}	76.68^{a}
Fresh weight (g)	1.61^{b}	3.21^{a}
Dry weight (g)	0.22^{b}	0.78^{a}

*Values in a row followed by the same letters indicate no significant differences between treatments at $P \leq 0.05$.

6.4 DISCUSSION

Glasshouse experiment

Control of ryegrass was significantly greater at week 6 compared to 3- and 9-week old ryegrass. A reasonable cause of such results would be differential mode of action of glufosinate ammonium at different weed growth stages. Glufosinate ammonium is dependent on the enzyme action of glutamine synthetase (Sellers et al. 2004). It is possible that when the plants are actively growing, absorption and translocation of glufosinate ammonium can be enhanced to increase efficacy. It can be argued that the same principle should apply for ryegrass at 3 weeks, but control of this relatively young stage was poor compared to both 6- and 9-week old plants. Leaf number, plant height and leaf area recorded showed that 6- and 9-week old plants were not significantly different from each other and both were significantly different from 3-week old plants (**Table 6.2**). Smaller leaf area for 3-week old plant might have reduced herbicide interception, thus reducing efficacy of herbicides (Steckelet al. 1997).

Response of ryegrass to control with the adjuvant Velocity® was much more pronounced when compared to glufosinate ammonium alone, or when using the adjuvant Summit Super®. The active ingredient in Velocity® is ammonium sulphate, whereas that of Summit Super® is a non-ionic nitrogen surfactant. The surfactant function of an adjuvant is to reduce the tension in the liquid in which it is dissolved (McMullan 2000). The sulphate ion in Velocity® is believed to react with the calcium cations to form calcium sulphate, thus allowing the ammonium ion to form readily absorbed NH_4-glyphosate molecule hence restricting the calcium cations from binding with the molecule (Pratt et al. 2009). The non-ionic Summit Super® active ingredient has the nitrogen source which helps glufosinate molecules move

effectively to the target site but lacks the anion which binds with magnesium or calcium on the leaf. The Velocity® mode of action on the leaf surface could be the reason why it controls ryegrass better than Summit Super®.

Field experiment

Ballista® and AMS both work as activator agents which increase herbicide activity. This experiment, however, did not show any statistical difference in control of ryegrass between glufosinate ammonium applied alone and with added Ballista®. According to Green (2002) activity of herbicides is influenced by the type of surfactant used. In some cases, adjuvants might not enhance herbicidal activity because their interaction differs with different herbicides and species (Stock and Holloway 1993; Hess and Chester 2000). Furthermore, studies have proven that the effect of alcohol ethoxylate surfactants differ based on the number of carbons in the structure (Green 2002). It is possible that effect of interaction of Ballista® with glufosinate ammonium and ryegrass did not effectively increase efficacy of glufosinate ammonium.

A dosage rate of 7.5 L ha^{-1} proved to be the best in controlling both young and mature ryegrass with mortality percentages ranging from 80 to 100%. However, considering the harsh conditions in the field, a control percentage of 75% young ryegrass with 5 L ha^{-1} glufosinate ammonium and AMS was relatively high. A reduction in efficacy of glufosinate ammonium observed on mature ryegrass can be explained by the temperature effect. Molefe (2015) observed better control of ryegrass at cool temperatures. Mature ryegrass was sprayed 4 weeks after the young grass when environmental temperature had also dropped, hence, this could have increased the efficacy of glufosinate ammonium (refer to **Table 6.4 and Table 6.5** for weather data).

6.5 CONCLUSION

Velocity® increased efficacy of glufosinate ammonium on ryegrass grown in the glasshouse. Addition of the adjuvant Summit Super® gave no significant increase in control of ryegrass compared to glufosinate ammonium without any additive. The field experiment showed that adjuvant AMS enhanced the efficacy of glufosinate ammonium on young ryegrass only. Ballista® did not show any significant improvement of ryegrass control compared to application of glufosinate ammonium only in young or mature ryegrass.

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CHAPTER 7

A COMPARATIVE STUDY OF GLUFOSINATE AMMONIUM EFFICACY ON RYEGRASS AND BAHIA GRASS AS INFLUENCED BY DIFFERENT TEMPERATURES

ABSTRACT

A glasshouse experiment was conducted at Welgevallen experimental farm to compare efficacy of glufosinate ammonium on two different grass species as influenced by temperature. The experiment was carried out on ryegrass (*Lolium multiflorum* cv Energa) and bahia grass *(Paspalum notatum* cv Pensacola*)*. Application of glufosinate ammonium was done 6 weeks after planting. Applied dosage rates were 1.5, 3, 4.5, 6 and 7.5 L ha^{-1}. The glasshouse temperatures were set at 10/15, 15/20, 20/25 and 25/30 °C night/day temperatures. Results showed a similar trend in glufosinate ammonium control of both grasses in which as temperature increased, control decreased. At 10/15 and 15/20 °C temperatures control was significantly higher compared to 20/25 and 25/30 °C temperatures. Even though the trend was similar, ryegrass was controlled to a lesser extent than bahia grass at higher temperatures.

Key words: bahia grass, dosage rate, glufosinate ammonium, ryegrass, temperature.

7.1 INTRODUCTION

Ryegrass has developed resistance to multiple herbicides through the processes of mutation and selection. Ryegrass is an out-crossing, open pollinated species and has great capability of undergoing hybridization hence it has higher levels of genetic variability (Ferreira et al. 2015). The recent introduction of herbicide resistant crops has provided an opportunity to increase the use of non-selective herbicides in controlling weeds (Green and Owen 2011; Vencill et al. 2012).

Glufosinate ammonium is a non-selective, post-emergent herbicide which inhibits the enzyme glutamine synthetase from degrading ammonia. This inhibition results in accumulation of ammonia which is phytotoxic to plant cells (Coetzer and Al-Khatib 2001; Sellers et al. 2004). Yellowing of plants is observed a few days after application followed by death (Petersen and Hurle 2000; Everman et al. 2009). However, not all weeds are effectively controlled by glufosinate ammonium despite its non-selective nature (Kumaratilake et al. 2002).

Differences in efficacy of glufosinate ammonium among species is accounted to the rate applied and extent of uptake and translocation (Mersey et al. 1990). Steckel et al. (1997) noted that absorption, translocation and plant metabolism contribute to the different plant sensitivities to herbicides. According to Kumaratilake et al. (2002), glufosinate ammonium action is rapid at the site of application hence translocation is minimum. This results in less effective control of annual and perennial weeds (Pline et al. 1999).

Poor control of rigid ryegrass as compared to *Avena* spp. has been observed in Australia (Kumaratilake et al. 2002). In addition to plant morphology and physiology, response to climatic conditions during and after herbicide application has an influence on efficacy of glufosinate ammonium (Archambault et al. 2001; Kumaratilake and Preston 2005; Penner 2015). A number of studies have proven that higher temperatures increases efficacy of glufosinate ammonium, however, inconsistencies among species have also been observed (Coetzer et al. 2001; Kumaratilake and Preston 2005). Molefe (2015) observed that glufosinate ammonium is less effective in controlling ryegrass at warmer temperatures than at cooler temperatures.

According to Bell et al. (2011), ryegrass is a temperate C_3 grass which grows under cool conditions while bahia grass is a tropical C_4 grass that grows under warm conditions (Houck 2009). Bahia grass does not pose weed problems but was included just as a tropical grass with seed that was easily obtainable. Therefore, the objective of this study was to observe and compare the efficacy of glufosinate ammonium in controlling bahia grass and ryegrass at different temperatures. It was hypothesized that glufosinate ammonium would be more effective on bahia grass at higher temperatures and vice versa for ryegrass, due to their contrasting temperature growth requirements.

7.2 MATERIALS AND METHOD

7.2.1 Experimental site

The experiment was conducted in a glasshouse at the Stellenbosch University Welgevallen Experimental Farm. The site is located at 33° 56´33" S and 18° 51´56" E and at an altitude of 136 m above sea level.

7.2.2 Treatments and experimental design

Commercial ryegrass (*Lolium multiflorum* cv Energa) and bahia grass (*Paspalum notatum* cv Pensacola) seeds were used in this experiment due to their ease of germination. A

randomized complete block design arranged as a 2×4×6 factorial with 5 replications was used for the experiment. The experimental factors were grass species at two levels (ryegrass and bahia grass), temperature at four levels (10/15, 15/20, 20/25 and 25/30 ºC night/day) and glufosinate ammonium dosage rates at six levels (0, 1.5, 3, 4.5, 6 and 7.5 L ha^{-1}).

7.2.3 Trial establishment and management

Planting

Seeds were first germinated in petri dishes in an incubator at 20 ºC under light conditions to ensure higher percentage germination and the resulting seedlings were transplanted into 8 x 8 cm square plastic pots after 2 weeks.

Fertilization

A nutrient solution was used to fertilize ryegrass plants during the study. The composition is shown **Table 7.1**.

Table 7.1: Composition of the nutrient solution used to fertilize the plants growing in pots

EC = 2.0			
Element (Macro)	Concentration mg L^{-1}	Fertilizer	Concentration g $1000L^{-1}$
K^{+}	237.7	KNO_3	303
Ca^{++}	180	K_2SO_4	261
Mg^{++}	48.6	$Ca(NO_3)_2.2H_2O$	900
NO_3^{-}	661.33	$MgSO_4.7H_2O$	492
H_2PO_4	116.4	KH_2PO_4	136
SO_4	390.4		
(Micro)	mg L^{-1}		
Fe	0.85	Libfer (Fe EDTA)	6.54
Mn	0.55	Manganese sulphate	2.23
Zn	0.30	Zinc sulphate	1.33
B	0.30	Solubor	1.46
Cu	0.05	Copper Sulphate	0.20
Mo	0.02	Sodium Molibdate	0.13

Weed, pest and disease control

Additional weeds were removed by hand. No pests and diseases were experienced in the glasshouse.

Irrigation

An automated irrigation system was used to water the plants. The plants were irrigated at 8:00 am, 12:00 pm, 2:00 pm and 4:00 pm. The quantity of water per irrigation was adjusted depending on the plant growth stage to compensate for water loss due to transpiration.

Herbicide application

Ryegrass and bahia grass were sprayed with glufosinate ammonium at 5-6 weeks after planting. The herbicide was applied five weeks after planting on the 9th of October 2015 by means of a pneumatic pot sprayer at a pressure of 2 bar in 200 L ha^{-1} of water. Evaluation was done 6 weeks after spraying.

7.2.4 Data collection

One set of control plants was harvested at the time of spraying and the following variables were recorded:

a. Number of leaves per plant

The number of leaves per plant were counted and recorded. The mean number of leaves of the four plants was then used as the number of leaves per experimental unit.

b. Leaf area (cm^2)

Leaf area per plant was measured using a leaf area meter (Li Cor) and the mean leaf area of the plants in a pot was then calculated.

c. Plant height (cm)

A calibrated ruler was used to measure plant height. The height considered was of the stems and leaves (above root) from the soil surface to the tip of the longest leaf of each plant. The mean plant height per pot was then calculated.

d. Wet biomass of live and dead matter (g)

From each pot, plants were harvested at the soil surface by means of secateurs and put into paper bags and the wet biomass was then measured using an electronic balance and recorded0. Wet biomass was expressed as biomass per pot.

e. Dry biomass of live and dead matter (g)

After determining the wet biomass, the paper bags with plants were put into an oven and dried at 80 ºC for 48 hours. The dry plants were then weighed on an electronic balance and the dry mass per pot was then calculated.

A period of six weeks allowed before evaluation was selected to ensure that no regrowth of the plants take place as was previously observed with glyphosate resistant ryegrass. The following variables was recorded:

a. Percentage mortality (%)

Percentage mortality was recorded six weeks after spraying. The calculation was done using the following formulae;

$$\text{Percentage mortality} = \frac{number\ of\ dead\ plants\ per\ pot}{4(plants\ per\ pot)} \times 100\%$$

b. Dry matter of surviving ryegrass expressed as percentage of the control plants

Green and live plants from treated pots were collected and dried. The dry matter of surviving ryegrass was calculated as a percentage of the unsprayed control plants. The calculation was done using the following formulae;

Dry matter of surviving ryegrass expressed as control percentage =

$$\frac{dry\ mass\ of\ surviving\ plants\ per\ pot\ at\ evaluation(g\ )\ \times\ 100\%}{average\ live\ dry\ matter\ per\ pot\ (g) of\ control\ plants}$$

7.2.5 Data analysis

Data were subjected to an analysis of variance using the STATISTICA 12 program. Means of significant main effects and interactions in the experiments were separated using Bonferroni test for control variables recorded at spraying time and Fischer's $LSD_{0.05}$ for the data variables recorded at evaluation (six weeks after spraying). Bonferroni confidence intervals for differences of the means are wider than that of Fisher's LSD, therefore, Bonferroni test was used for one-way ANOVA of control variables whilst Fischer's $LSD_{0.05}$ was used for the factorial ANOVA.

7.3 RESULTS

A three-way interaction (P = 0.020129) occurred between factors in terms of percentage control (See ANOVA table in Appendix 15; **Figure 7.1**). According to **Figure 7.1,** 100% control of ryegrass and bahia grass was observed at 10/15 and 15/20 °C temperatures when sprayed with glufosinate ammonium dosage rates of 3, 4.5, 6 and 7.5 L ha^{-1}. Complete (100%) control of ryegrass using a dosage rate of 6 and 7.5 L ha^{-1} was observed at 20/25 °C, while at 25/30 °C only the 7.5 L ha^{-1} dosage rate gave 100% control. A decrease in the control of both bahia grass and ryegrass occurred at the two higher temperatures, most

notably at 25/30 °C with the lower dosage rates. However, even though control of both grasses decreased as temperature increased, ryegrass control was more negatively influenced by the higher temperatures than bahia grass e.g. at 4.5 L ha^{-1} dosage rate at 25/30 °C bahia grass displayed significantly higher control percentage (85%) compared to ryegrass (40%).

There was a significant interaction of grass species, temperature and dosage rate on dry matter of live plants expressed as percentage control with a p value of 0.00 (See ANOVA table in appendix 16). Percentage dry matter of surviving plants (**Figure 7.2**) showed that at 10/15, 15/20 and 20/25 °C at least 95% control was achieved in ryegrass at 3 L ha^{-1} while in bahia grass 100% control was achieved at 10/15 and 15/20 °C temperatures only using the same dosage rate. It required 6 L ha^{-1} dosage rate to control bahia grass 100% at 20/25 °C and even at 7.5 L ha^{-1} there was not 100% control. In general, dry matter percentage was higher at 20/25 and 25/30 °C temperatures proving that control were poor at high temperatures. This was, however, only statistically significant at the 1.5 L ha^{-1} dosage rate.

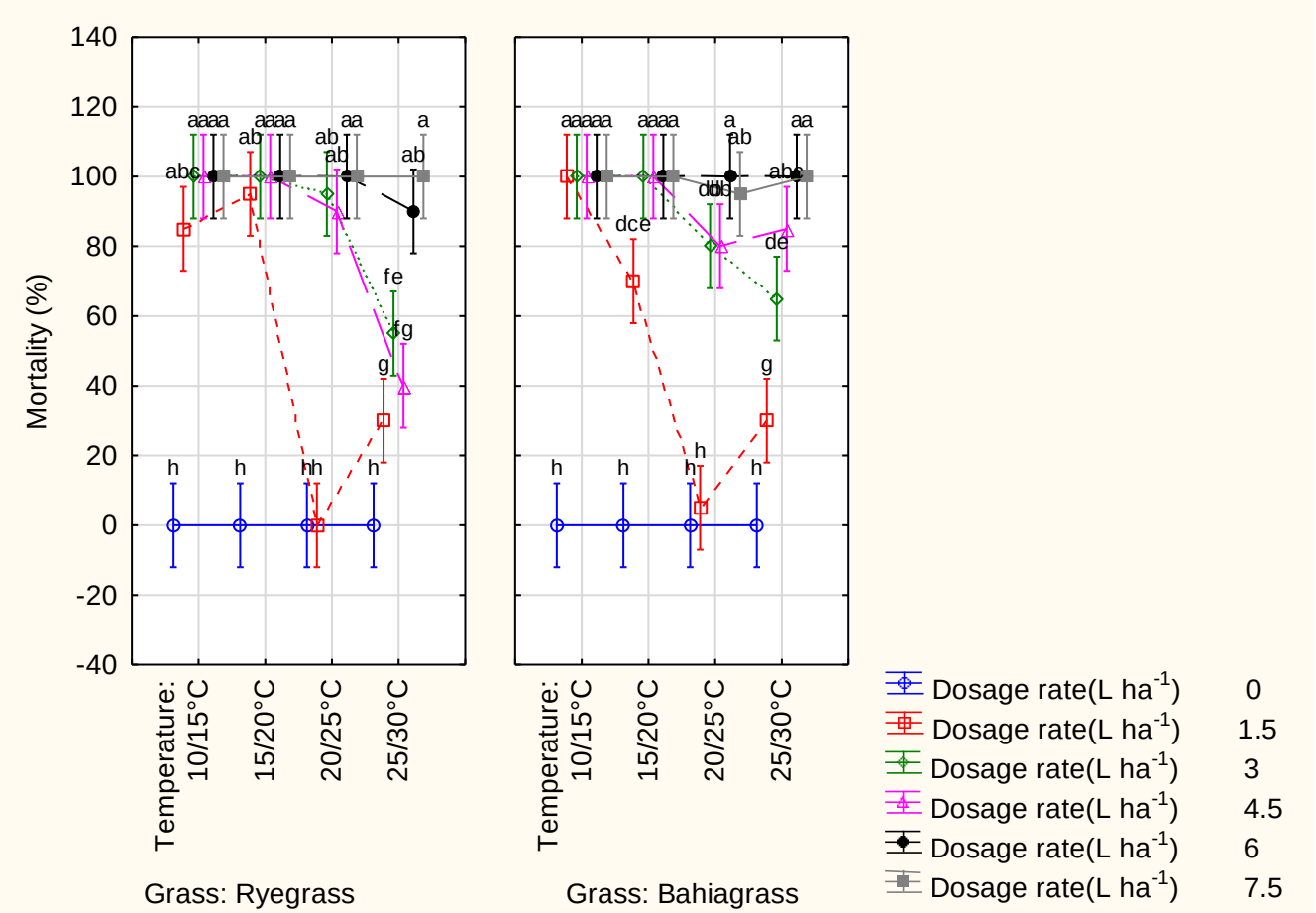

Figure 7.1: Mortality rates of ryegrass and bahia grass after application of various glufosinate ammonium dosage rates at four different temperature regimes. Means indicated by different letters differed significantly at $P \leq 0.05$ according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

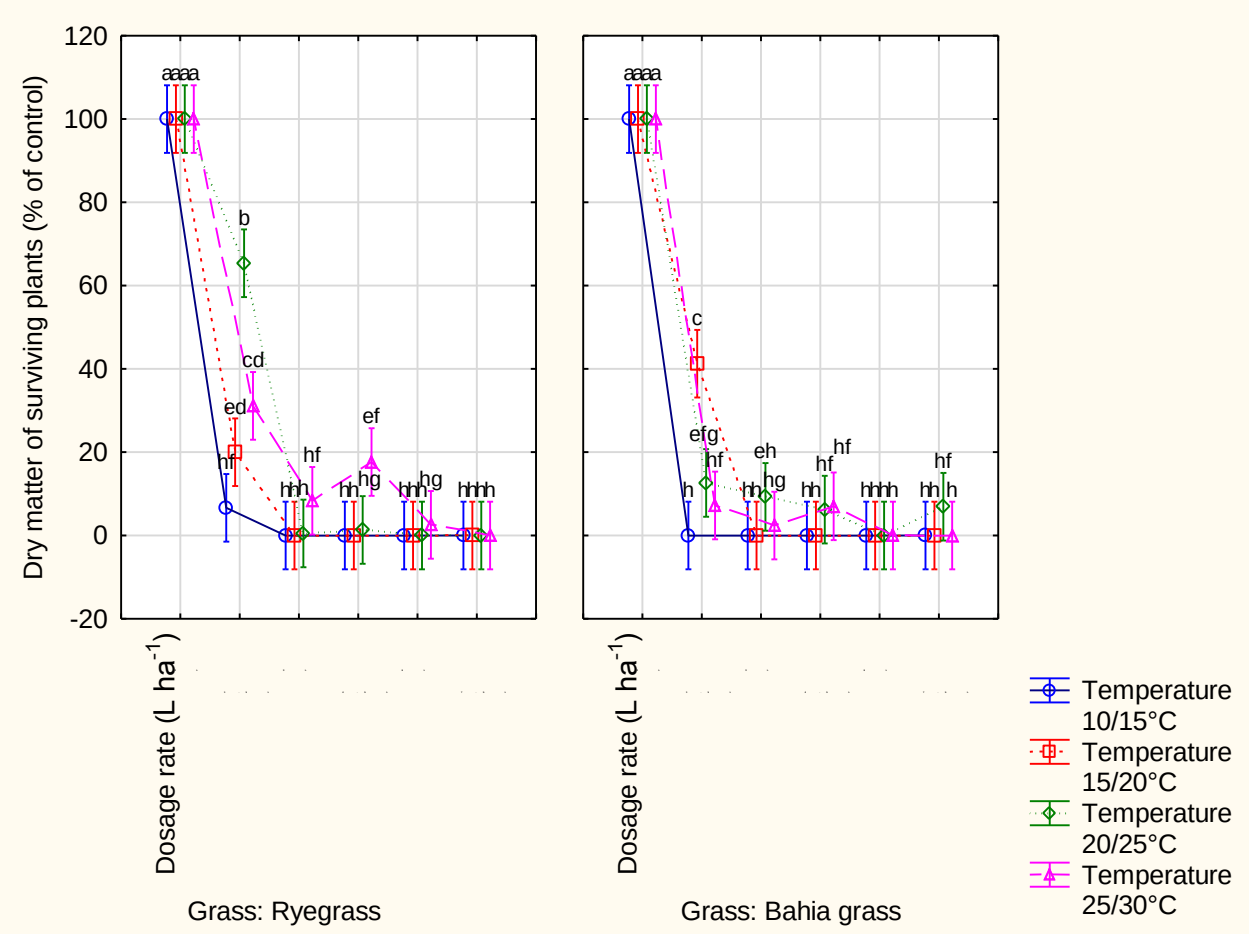

Figure 7.2: Dry matter of surviving ryegrass and bahia grass expressed as a percentage of the unsprayed control after application of various glufosinate ammonium dosage rates at four different temperature regimes. Means indicated by different letters differed significantly at P ≤ 0.05 according to Fishers protected LSD. Vertical bars denote 95% confidence intervals.

Table 7.2: Vegetative growth parameters of control bahia grass plants growing at different temperatures at the time of spraying

Bahia grass variables	**TEMPERATURE**			
	10/15 °C	**15/20 °C**	**20/25 °C**	**25/30 °C**
Leaf number*	4^{b}	4^{b}	5^{a}	6^{a}
Plant height(cm)	5.66^{c}	5.98^{c}	14.28^{b}	31.02^{a}
Leaf area(cm^2)	7.83^{b}	7.31^{b}	7.34^{b}	14.51^{a}
Fresh weight(g)	0.23^{c}	0.92^{b}	0.82^{b}	2.32^{a}
Dry weight (g)	0.04^{b}	0.066^{b}	0.24^{ab}	0.33^{a}

*Values in a row followed by the same letters indicate no significant differences between treatments at $P \leq 0.05$.

Table 7.3: Vegetative growth parameters of control ryegrass plants growing at different temperatures at the time of spraying

Ryegrass variables	**TEMPERATURE**			
	10/15 °C	**15/20 °C**	**20/25 °C**	**25/30 °C**
Leaf number *	8^{ab}	9^{a}	6^{b}	8^{ab}
Plant height(cm)	17.58^{b}	25.58^{a}	26.29^{a}	26.19^{a}
Leaf area(cm^2)	13.98^{c}	25.95^{a}	19.76^{b}	24.82^{ab}
Fresh weight(g)	2.14^{b}	4.48^{a}	2.65^{b}	2.82^{b}
Dry weight (g)	0.57^{b}	0.68^{a}	0.48^{b}	0.48^{b}

*Values in a row followed by the same letters indicate no significant differences between treatments at $P \leq 0.05$.

7.4 DISCUSSION

An increase in temperature results in a transient increase of cytoplasmic calcium ion concentration (Gong et al. 1998; Knight 2000; Knight and Knight 2001). Exposure to high temperatures of tobacco (*Nicotiana plumbaginifolia*) induced increase in cytoplasmic Ca^{2+}. Calcium ions reacts with the glufosinate molecule to form a less soluble compound. It could be possible that the continuous exposure of ryegrass and bahia grass to high temperatures such as 20/25 and 25/30°C led to a permanent high concentration of calcium ions. The concentration could have been high enough to significantly reduce the efficacy of glufosinate ammonium at higher temperatures as compared to lower temperatures.

By considering leaf number, leaf area and dry weight, optimum growth of ryegrass in this experiment was observed at temperatures 15/20 °C whilst that of bahia grass was observed at 25/30 °C. Dry matter accumulation of bahia grass proved that growth increased as temperature increased. Bahia grass is a C_4 grass which grows best under high temperatures and acidic soils (Houck 2009). It is likely that lower temperatures (10/15 and 15/20 °C) and the standard solution with neutral pH were unfavorable conditions for bahia grass, hence, the grass was under stress. It could be expected that under stress, bahia grass would be difficult to control since stressed plant decrease efficacy of herbicides (Ahmadi et al. 1980; Steckel et al. 1997). However, this study gave contrary results to literature, with significantly greater control of bahia grass at 10/15 and 15/20 °C than at warmer temperatures.

Contrary to bahia grass, ryegrass is a temperate grass which grows best under cool temperatures. However, optimum growth was observed at 15/20 °C, while ryegrass growth at 20/25 and 25/30 °C was relatively high. Expected growth observations would have been optimum growth at 10/15 and 15/20 °C, instead, poor growth was recorded at 10/15 °C as compared to the rest of the temperatures. Growth of ryegrass at 10/15 °C was significantly poorer than that of ryegrass at 20/25 in terms of all parameters recorded. The reason for this is unclear. Regardless of the above-mentioned observations, control of ryegrass was greater at lower temperatures than higher temperatures. The most reasonable explanation would be accumulation of calcium ions because of continuous exposure to high temperatures and/or the development of a thicker waxy cuticle in planrs growing at higher temperatures.

Glufosinate ammonium showed the same trend in controlling both grasses in which mortality rate was higher at lower temperatures than at higher temperatures. For both grasses, the trend of results might be attributable to increased calcium accumulation as temperature increases (Clapham 2007).

7.5 CONCLUSION

Results suggested that mortality rates shown by glufosinate ammonium on ryegrass were similar to that for bahia grass. There was a general decrease in control of both grasses as temperature increased. The possible role of calcium concentration in leaf tissue on glufosinate ammonium efficacy deserves investigation.

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CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusion

In countries where herbicide-resistant crops have been introduced, adoption of glufosinate ammonium as a post-emergence herbicide is relatively low compared to a herbicide such as glyphosate (Molefe 2015). Inconsistent control of glufosinate ammonium might also impose this kind of response from farmers since effective control of weeds is not guaranteed. However, with the increasing resistance of ryegrass weeds to commonly used herbicides, glufosinate ammonium could be a possibility that could either replace or be applied alternatively with the commonly used herbicides like glyphosate or paraquat, in conditions where non-selective herbicides can be applied.

This study has established that glufosinate ammonium efficacy was high at relatively low temperatures (10/15 and 15/20 °C night/day). At higher temperatures, control of ryegrass required higher dosage rates of glufosinate ammonium to reach a mortality of 100%. The same trend was observed for bahia grass. Field experiments with adjuvants (objective D2) appear to have supported the glasshouse findings, since control of mature ryegrass with glufosinate ammonium under cooler environmental conditions was significantly better on young ryegrass compared to control at warmer temperatures.

Numerous studies have proved that weeds are most easy to control when they are young. In contrast, the current field studies found no significant difference in the control of ryegrass at different growth stages. Instead, in most cases, control seemed to improve as growth stage increased, although differences were not always statistically significant. Glasshouse experiments gave a slightly different shift where greater control was observed for 6-week old ryegrass. However, control of 9-week old and 10-week old ryegrass was more effective compared to young 2- and 3-week old ryegrass.

Addition of adjuvants to herbicides are well-known for enhancing efficacy. Findings of this study confirmed that AMS (ammonium sulphate), both in a ready mixed liquid formulation marketed as Velocity®, and as granules dissolved in water to form a 1, 2 or 3% solution, enhanced efficacy of glufosinate ammonium more than the non-AMS adjuvants, Ballista® and Summit Super®. Control of ryegrass was better at lower temperatures than at higher temperatures, even in the presence of AMS adjuvant. A 3% AMS solution resulted in the highest mortality of ryegrass. In the field, AMS enhanced control of young ryegrass but

not of mature ryegrass. Control of ryegrass with Ballista® was not significantly different to that of glufosinate ammonium alone.

Possible explanations of the observed results include the influence of both environmental and plant factors. Increase in temperature results in the production of a waxy layer in leaves which results in a thick cuticle, thus reducing both absorption and translocation of glufosinate ammonium (Jamal 2011). It has been reported that higher temperatures also increase calcium concentration in the leaves, which in turn react with the glufosinate molecule resulting in a less soluble substance and reduced efficacy (Pratt et al. 2003). Addition of AMS will result in the ammonium ion reacting with glufosinate ammonium giving a more soluble compound, hence, increasing absorption and translocation of the herbicide. In hard water, or water of poor quality as regards the presence of cations such as Ca and Mg, the sulphate ions contributed by AMS will react with those cations, thus preventing their antagonistic chemical reaction with herbicide molecules.

8.2 Recommendations

The western Cape predominantly receives its rainfall in winter consequently providing favourable conditions for the growth of ryegrass. The average winter temperature ranges from 10 to 19 °C during this period. At such temperatures, the study revealed that glufosinate ammonium will likely show effective control of ryegrass. This makes glufosinate ammonium a suitable alternative in alleviating ryegrass resistance in the western Cape. However, more research needs to be done for a wider range of climates, especially in field conditions. The temperature study suggested roles for calcium accumulation in leaves and wax deposit on the plants as temperature increased. At this stage these represent unsubstantiated conjecture. More research to prove these theories are needed.

Field experiments showed that it is important to consider environmental conditions before and at the time of applying glufosinate ammonium. Soil seedbank of weeds was of high importance at Langgewens experimental farm. It would be advisable to apply the herbicide after most of the seed has germinated to avoid poor control. This study proved that growth stage of ryegrass has no influence on efficacy of glufosinate ammonium, hence, the herbicide can be applied when much of the dormant seed has germinated, provided it is in row crops or herbicide-resistant crops to ensure no crop damage. However, the critical stage at which crops are likely to be affected most by competing weeds should be taken into consideration. By considering both periods, i.e. the critical stage of weeds causing yield loss in crops, and

time when most seeds have germinated, the right time to apply the herbicide can be determined, not only with the purpose of achieving high crop yields but also to control a higher percentage of weeds.

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APPENDICES

Appendix 1: Analysis of variance on ryegrass mortality in the glasshouse at different temperatures and growth stages

Effect	Fixed Effect Test for % mortality		
	Degree of freedom	F-value	P-value
Temperature	3	26.015	≤0.0001
Age	1	31.441	≤0.0001
Dosage rate	5	182.309	≤0.0001
Temperature*Age	3	7.988	≤0.0001
Temperature*Dosage rate	15	6.346	≤0.0001
Age*Dosage rate	5	3.626	0.00371
Temperature*Age*Dosage rate	15	3.566	≤0.0001

Appendix 2: Analysis of variance on dry matter of live plants expressed as percentage of the unsprayed control in the glasshouse at different temperatures and growth stages

Univariate tests of significance for dry matter of surviving ryegrass (% of control)					
Effect	SS	DF	MS	F	P-value
Intercept	11,236	1	11,237	1373,934	≤0.0001
Temperature	1,022	3	0,341	41,665	≤0.0001
Age	0,704	1	0,704	86,127	≤0.0001
Dosage rate	23,965	5	4,793	586,061	≤0.0001
Temperature*age	0,197	3	0,066	8,019	≤0.0001
Temperature*dosage rate	1,482	15	0,099	12,079	≤0.0001
Age*dosage rate	0,410	5	0,082	10,031	≤0.0001
Temperature*Age*Dosage rate	1,068	15	0,071	8,707	≤0.0001
Error	1,570	192	0,008		

Appendix 3: Analysis of variance on ryegrass mortality in the glasshouse at different temperatures and AMS concentrations

Effect	Fixed Effect Test for % mortality		
	Degree of freedom	F	P-value
Temperature	3	10.184	≤0.0001
Dosage rate	2	66.146	≤0.0001
AMS concentration	2	6.954	0.001233
Temperature*Dosage rate	6	1.491	0.183382
Temperature*AMS concentration	6	5.265	≤0.0001
Dosage rate*AMS concentration	4	2.992	0.020149
Temperature*dosage rate*AMS concentration	12	2.273	0.010420

Appendix 4: Analysis of variance on dry matter of live plants expressed as percentage of the unsprayed control in the glasshouse at different temperatures and AMS concentrations

Effect	Fixed Effect Test for dry matter of surving ryegrass(g)		
	Degree of freedom	F	P-value
Temperature	3	31,375	≤0.0001
Dosage rate	2	37,980	≤0.0001
AMS concentration	2	8,635	0,000262
Temperature*Dosage rate	6	17,324	≤0.0001
Temperature*AMS concentration	6	7,104	≤0.0001
Dosage rate*AMS concentration	4	5,629	0,000272
Temperature*Dosage rate*AMS concentration	12	6,47	≤0.0001

Appendix 5: Analysis of variance of ryegrass mortality on different growth stages in the glasshouse that were planted once

Effect	Fixed Effect Test for % mortality method 1-planted once		
	Degree of freedom	F	P-value
Growth stage	4	1,797	0,133940
Dosage rate	5	153,18	≤0.0001
Growth stage*Dosage rate	20	3,485	≤0.0001

Appendix 6: Analysis of variance on dry matter of live plants expressed as percentage of the unsprayed control at different growth stages - method 1 in the glasshouse

Effect	Fixed Effect Test for dry matter of surviving plants (% of control)		
	Degree of freedom	F	P-value
Growth stage	4	3,191	0,016
Dosage rate	5	176,3	≤0.0001
Growth stage*Dosage rate	20	3,895	≤0.0001

Appendix 7: Analysis of variance of ryegrass mortality on different growth stages in the glasshouse that were sprayed once

Effect	Fixed Effect Test for % mortality method 2-sprayed once		
	Degree of freedom	F	P-value
Growth stage	4	10,475	≤0.0001
Dosage rate	5	178,998	≤0.0001
Growth stage *Dosage rate	20	9,097	≤0.0001

Appendix 8: Analysis of variance on dry matter of live plants expressed as percentage of the unsprayed control at different growth stages - method 2 in the glasshouse.

Effect	Fixed Effect Test for dry matter of surviving ryegrass (% of control)		
	Degree of freedom	F	P-value
Growth stage	4	6,586	≤0.0001
Dosage rate	5	430,128	≤0.0001
Growth stage*Dosage rate	20	3,177	≤0.0001

Appendix 9: Analysis of variance of ryegrass mortality on different growth stages at Welgevallen experimental farm

Effect	Percentage mortality		
	Degree of freedom	F	P-value
Week	4	8.976	≤0.0001
Dosage rate	4	222.414	≤0.0001
Week*Dosage rate	16	1.648	0.077

Appendix 10: Analysis of variance of ryegrass mortality on different growth stages at Roodebloem experimental farm

Effect	Percentage mortality		
	Degree of freedom	F	p
Week	4	17.190	≤0.0001
Dosage rate	4	447.034	≤0.0001
Week*Dosage rate	16	14.644	≤0.0001

Appendix 11: Analysis of variance of ryegrass mortality at Langgewens experimental farm at different growth stages

Effect	Percentage mortality				
	SS	Degree of Freedom	MS	F	p
Week	28085.8	4	7021.5	35.705	≤0.0001
Dosage rate	79412.4	4	19853.1	100.957	≤0.0001
Week*Dosage rate	16621.6	16	1038.8	5.283	≤0.0001
Error	14748.7	75	196.6		

Appendix 12: Analysis of variance on different growth stages of ryegrass mortality in the glasshouse using glufosinate ammonium with added adjuvants

Effect	Fixed Effect Test for % mortality		
	Degree of freedom	F	P-value
Growth stage	2	32	≤0.0001
Dosage rate	5	273,843	≤0.0001
Adjuvant	2	11,498	≤0.0001
Growth stage *Dosage rate	10	10,366	≤0.0001
Growth stage*Adjuvant	4	4,303	0,002
Dosage rate*Adjuvant	10	5,97	≤0.0001
Growth stage*Dosage rate*Adjuvant	20	2,307	0,002

Appendix 13: Analysis of variance on dry matter of live plants expressed as percentage of control using glufosinate ammonium with added adjuvants in the glasshouse at different growth stages

Effect	Fixed Effect Test for dry matter of surviving ryegrass (% of control)		
	Degree of freedom	F	p
Growth stage	2	8,528	0,0003
Dosage	5	439,282	≤0.0001
Adjuvant	2	9,244	0,0001
Growth stage*Dosage rate	10	3,651	0,0001
Growth stage*Adjuvant	4	3,362	0,011
Dosage rate*Adjuvant	10	3,090	0,001
Growth stage*Dosage rate*Adjuvant	20	1,522	0,076

Appendix 14: Analysis of variance on different growth stages of ryegrass mortality at Welgevallen experimental farm using glufosinate ammonium with added adjuvants

Effect	Fixed Effect Test for % mortality		
	Degree of freedom	F	p
Growth stage	1	4,446	0,038
Dosage rate	3	134,232	≤0.0001
Adjuvant	2	1,628	0,203
Growth stage*Dosage rate	3	2,223	0,093
Growth stage*Adjuvant	2	1,279	0,284
Dosage rate*Adjuvant	6	2,465	0,032
Growth stage*Dosage rate*Adjuvant	6	1,081	0,382

Appendix 15: Analysis of variance on ryegrass and bahia grass mortality in the glasshouse at different temperatures

Effect	Percentage mortality		
	Degree of freedom	F	P-value
Temperature	3	49,585	≤0.0001
Grass	1	0,503	0,478
Dosage rate	5	321,600	≤0.0001
Temperature*Grass	3	4,084	0,008
Temperature*Dosage rate	15	17,070	≤0.0001
Grass*Dosage rate	5	0,839	0,523
Temperature*Grass*Dosage rate	15	1,958	0,020

Appendix 16: Analysis of variance on dry matter of live bahia grass and ryegrass expressed as a percentage of the unsprayed control in the glasshouse at different temperatures

Effect	Univariate Tests of Significance for dry matter of surviving ryegrass (% of control)				
	SS	Degree of Freedom	MS	F	P-value
Intercept	11,406	1	11,406	1345,513	≤0.0001
Temperature	0,203	3	0,068	8,002	≤0.0001
Grass	0,038	1	0,038	4,517	0,035
Dosage rate	30,81	5	6,162	726,908	≤0.0001
Temperature*Grass	0,103	3	0,034	4,039	0,008
Temperature*Dosage rate	0,65	15	0,043	5,111	≤0.0001
Grass*Dosage rate	0,208	5	0,042	4,918	0,0002
Temperature*Grass*Dosage rate	0,69	15	0,046	5,420	≤0.0001
Error	1,628	192	0,008		